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THE EVOLUTION OF THE PEDICULATE FISHES

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THE medieval imagination, rioting in strange imps and hobgoblins, could hardly have invented anything more malevolent in appearance than the ceratioids or deep-sea angler-fishes, sometimes called sea devils. Naturally enough, these black sea devils live in the kingdom of darkness, where they prowl about seeking whom they may devour. Many of them even carry a sort of torch, illumined with a phosphorescent glow, with which they lure their victims within reach of their devouring jaw-traps.

It may well be realized that the middle layers of the ocean, from about 500 to 2,000 meters below the surface, where the deep-sea anglers dwell, have not yielded up their most fantastic inhabitants without long and systematic trawling by oceanographic expeditions. These have slowly amassed the collections described by Günther, Lütken, Garman, Brauer, Regan and Trewavas, Parr, Beebe and others. A recent monograph by C. Tate Regan and Ethelwynn Trewavas deals with the ceratioids of the Carlsberg Foundation's Oceanographical Expedition, under the leadership of the late Professor Johannes Schmidt. It contains very full and satisfactory accounts of the anatomy, osteology and classification of the families of Ceratioidea, and has been our principal source for that division of the order Pediculati. We have ourselves also studied the construction of the skull in *Lophius, An-*

tennarius, Ogcocephalus, and through the kindness of Dr. William Beebe have had the opportunity of examining preparations of several ceratioids.

Regan and Trewavas (1932, p. 25) divide the order Pediculati into three suborders: the Lophioidea (including the ordinary anglers and the sea-bats), the Antennarioidea (including chiefly the sea-mice) and the Ceratioidea, or deep-sea anglers.

In our studies which have provided the data for the present summary we have constantly had before us the following questions:

(1) Assuming as we must, for many reasons, that the pediculates are merely very highly specialized offshoots of the spiny-finned stock of teleost fishes, what have been the main steps during the transformation of a primitive perch-like fish into the least specialized among the known pediculates?

(2) What were the principal lines of cleavage leading respectively to the true anglers (*Lophius*), the sea-bats (ogcocephalids), the sea-mice (antennariids) and the deep-sea anglers (ceratioids)?

(3) Assuming the taxonomic interrelationships of the families of ceratioids to be as determined by Regan and Trewavas, can we arrange figures of representatives of the various families in such a way as to indicate the probable phylogenetic relationships?

(4) By what steps have the "parasitic males" of the ceratioids attained their present high specializations?

In order to save space we shall attempt to combine our tentative answers to these questions so as to present in outline our interpretation of the evolution of the entire order.

The outstanding features of a typical pediculate are as follows: (1) its illicium, or lure; (2) its immense mouth cavity and throat; (3) the peculiar nature of the exit from the gill chamber, the usual slit behind the gill-cover of either side being replaced by a slit or pore which opens behind the base of the pectoral fin; (3) the "pediculate"

character of the pectoral fins, which are also typically geniculate, that is, with an elbow-like joint by means of which they can be turned downward or upward.

As to the evolution of the illicium, the most elementary comparative observations were long ago sufficient to show that this structure is really a highly specialized product of the dorsal fin (Fig. 1). More precisely, the stages of

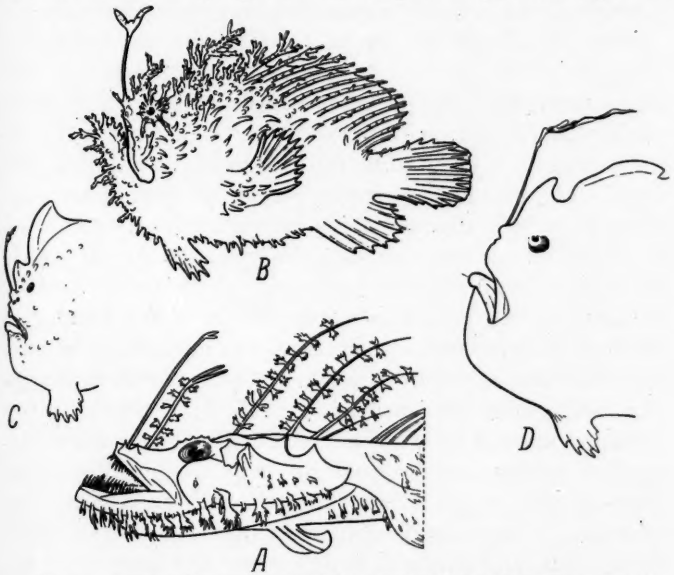

FIG. 1. Comparative series of illicia, showing their derivation from the dorsal fin.

A. *Chirolophius naresii.* After Boulenger.
B. *Rhycherus filamentosus.* After McCulloch and Waite.
C. *Antennarius leprosus.* After Jordan and Evermann.
D. *Antennarius laysanius.* After Jordan and Evermann.

its derivation appear to have been somewhat as follows: (1) In a somewhat remote ancestral form the dorsal fin became elongate and some of its anterior or spiny-rayed part extended forward over the top of the skull, finally reaching the snout. Each of these spiny rays was supplied with its own bony basal support and with small paired muscles for raising or lowering the fin-ray. (2)

Bits of skin near the tops of the first few fin-rays began to assume some functional importance as a lure for small fishes, perhaps due to their being shaken as the fish lurked in the seaweed. Such a stage is seen in *Chirolophius*. (3) Originally the first three rays participated in this function, but finally the first ray alone became specialized into an illicium, or lure, the rays immediately behind it being eventually suppressed entirely.

The subsequent history of the illicium was evidently different in the different families of ceratioids. In the Photocorynidae the illicium became very short and practically sessile. In one species of the Gigantactinidae, on the contrary, it grew to be four times as long as the fish itself. The basal bone supporting the illicium in extreme cases grew long and slender, protruding from the top of the snout like a long fishing pole, from the end of which the long illicium trailed backward, as in *Lasiognathus*. In some of the parasitic males, on the other hand, the illicium disappeared. In the anglers (lophiids) and seamice the free end of the illicium is covered with ordinary skin but among the ceratioids (Fig. 2) the end of the illicium varies from a simple bulb containing a dark pigmented nucleus surrounded by pale tissue (*Mancalias uranoscopus*) to an extraordinarily elaborate structure, which in *Chaenophryne fimbriata* may simulate a luminous shrimp. Nothing is known about the ways in which their owners use these strange lures but since they evidently capture living things by a sudden snap of the jaws, it can hardly be doubted that the lures are of value in enabling the fish to get close to its prey.

In typical pediculates the mouth cavity is of enormous capacity, so that the fish can engulf correspondingly large prey. The great increase in the size of the mouth cavity as compared with that in an ordinary fish has been brought about by increase in three directions: transverse, vertical and anteroposterior. Evidence of increase in the transverse plane across the roof of the mouth is seen in the great relative width, especially of the rostral part of

FIG. 2. Various illicia of deep-sea pediculates.

A. *Dolopichthys simplex.* C. *Himantolophus danae.*
B. *Dolopichthys microlophus.* D. *Chaenophryne fimbriata.*

All after Regan and Trewavas, 1932.

the skull in such forms as *Melanocetus, Paroneirodes, Himantolophus, Chaenophryne,* etc. Increased vertical diameters of the mouth cavity have been secured by elongating the suspensorium (hyomandibular plus symplectic plus quadrate) so as to depress the level of the posterior part of the mandible. An increased anteroposterior diameter of the mouth cavity has been brought about occasionally by lengthening the floor of the skull anteroposteriorly, as in *Cryptosparus* and *Gigantactis;* but by far the greatest increase has been in the anteroposterior dimensions of the bones forming the floor and sides of the branchial region. In general: (a) a long and forwardly inclined suspensorium together with a short muzzle tend to make the jaws point upward (as in *Melanocetus* and *Cryptosparas*); (b) a long backwardly inclined suspensorium (as in *Caulophryne*) or a short muzzle (as in *Gigantactis*) tends to direct the mouth forward.

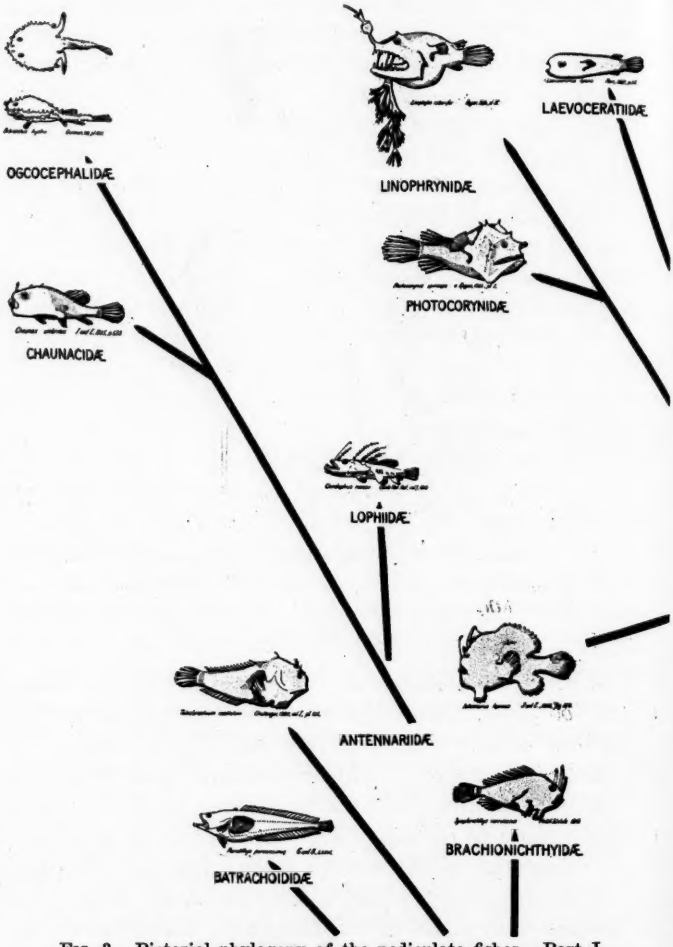

FIG. 3. Pictorial phylogeny of the pediculate fishes. Part I.

In ordinary fishes there is of course a deep fissure, the respiratory cleft, between the posterior edge of the opercular flap covering the gill chamber and the anterior edge of the crescentic shoulder-girdle. This cleft is usually continued below to the isthmus or median part beneath the basibranchial bones. The lower part of the opercular

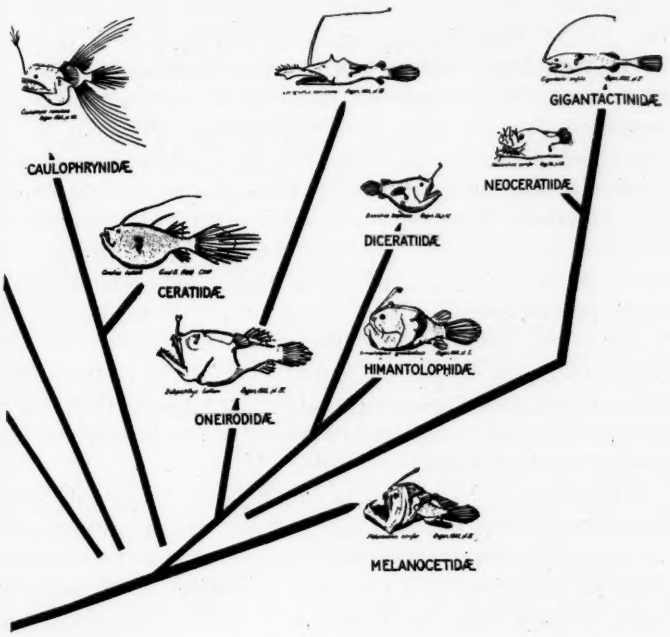

FIG. 4. Pictorial phylogeny of the pediculate fishes. Part II.

flap is supported by the curved branchiostegal bones, which are attached below to the two main lower segments of the hyoid arch. In the pediculates there has been an enormous increase in the size of the opercular flap and the branchiostegal bones have become very long and much curved. This great opercular-branchiostegal flap now overlaps the shoulder-blade, and its skin has become confluent with that which covers the cleithrum. The result is that the opercular or respiratory cleft no longer appears in its customary place but has moved far backward and is represented by a small opening which in most

pediculates lies below and behind the axilla of the pectoral fin. Partly transitional conditions may be seen in the batrachoid fishes, which show an early stage in the expansion of the branchiostegal flap. In *Tetrabrachium* the branchiostegal membrane is apparently connected with the axillary region of the pectoral fin. In *Lophius* there is on each side a rather narrow passageway leading from the enormous oral cavity to the respiratory opening, which is beneath and somewhat behind the axilla of the pectoral fins. Similar conditions may be observed in most other pediculates.

These respiratory passages, which are known to act like syringes in the sea-mice, may possibly be of some use in forward propulsion, especially if the fish is distended with a full meal; and also in view of the feebleness of the pectoral fins in many ceratioids.

The enormous dilatation of the throat in typical pediculates has left the pectoral fins high up on the sides of the body and in the more specialized ceratioids in a much reduced condition. In the more primitive antennariids, however, the high functional value of the pediculate fins is conspicuous. In the living mouse-fish of the Sargasso Sea (*Pterophryne*) one could see the large paw-like pectoral fins being turned downward so that they could extend beneath the huge throat and clasp the fronds of seaweed, along which the fish walked by alternately moving his pectoral and pelvic "feet." At other times the pectoral paddle, by virtue of its elbow-like joint, was turned upward in seeking contact with the fronds that were somewhat above the horizontal line of the fish. This movement was described by one of us in "Studies on the Body-form of Fishes"[1] as follows:

> The third series of observations was made by Mr. Dwight Franklin and myself on living specimens of the Sargasso fish (*Pterophryne histrio*) in aquaria on board the "Arcturus." *Pterophryne* is a short and fairly deep, thick-set, carnivorous little fish, with a very small upturned mouth and great hand-like pectoral fins with movable elbows; it has a prominent backwardly extended dorsal fin and downwardly projecting ventral fins that end below

[1] *Zoologica*, March 1, 1928, Vol. VIII, No. 6, pp. 408–9.

in large white "feet." Its golden-brown ground-color with irregular patches of dark brown, flecked with little white circular spots, forms a perfect camouflage as the fish lurks on the gold and brown weeds.

When swimming slowly the principal thrusts were caused by the rhythmic jets of water from the small rounded gill openings, modified by the gentle undulations of the pectoral fin membranes.

When crawling along the branches of the weed the *Pterophryne* sometimes moved as if it were stalking the alert little fishes and crustaceans upon which it feeds. One long pectoral flipper would be slowly swung forward while the opposite one was moving backward, the body being supported below by the large white feet, which turned outward and shuffled away in the well known manner of the cinema comedian.

When resting in the weed the fish maintained his position with all four paired fins and with as many median fins as could reach parts of the weed. One huge pectoral "arm" would be extended almost straight upward, the finger-like tips of the dermal rays clutching a branch of the weed that hung down above the fish; the opposite pectoral was thrust downward and reflected at the "wrist," the "palm" turned outward and forward and the palmar side of the "fingers" touching weed. One long "foot," following another branch of weed, was cocked forward and upward; the other, reaching still another branch, was directed backward and downward. The posteriorly elongate part of the dorsal was folded over and served as another prop, and at other times the caudal and anal fins also cooperated in keeping the fish securely placed in spite of the movements of wind or wave.

But it would be a mistake to infer that *Pterophryne* was always a sluggish, slow moving fish. When one of these fishes was placed in a large pan and attempts were made to catch it by hand, it made great flying leaps, such as it may have made in sudden dashes after its prey or in overtaking the weed after brief excursions.

The pediculate character of the pectoral is due to the circumstance that its "radial" bones or pterygials are elongate, so that the fleshy base is extended. The geniculate or elbow-like movement is possible partly because there is a curved articular surface along the line of contact between the base of the fin rays and the supporting "radials," permitting a wide range of movement of the "hand" upon the "wrist," and because this articular surface is in itself rather sharply inclined to the long axis of the "wrist." Moreover, the whole fin can be drawn and twisted downward toward the "chest" or upward toward the back. The main articular surface of the pectoral fin is often borne on one elongate "radial" which has an expanded distal articular end and may, purely for present descriptive purposes, be called the "ulna"; another and

smaller "radial" lies on the upper border of the base of the fin and may here be called the "radius." The "radius" and the "ulna" seem to be constant in most pediculates, but inspection of the figures of Regan and Trewavas and other sources shows that between the "ulna" and the "radius" we find either two rods (in the Melanocetidae, Gigantactinidae, Ceratiidae), or one rod (in the Antennariidae, Chaunacidae, Diceratiidae, Himantolophidae, Oneirodidae, Photocorynidae, Linophrynidae), or none at all (in the Lophiidae, Ogcocephalidae, Caulophrynidae). After prolonged consideration and comparison we think it probable: (a) that the most primitive condition is to be found in the Antennariidae and others that have but one extra rod between the "radius" and the "ulna"; (b) that the additional rods in those families with four "radials" have arisen by longitudinal splitting of the "ulna"; and (c) that the smallest number (2) has arisen by fusion of the intermediate rod with the "ulna."

With regard to the general form of the body there seems good reason to believe that the diversified ceratioids, which are for the most part huge floating traps with small pectorals and no pelvic fins, have been derived from more active forms like the sea-mice (antennariids) with strongly developed pectoral and pelvic fins. In *Antennarius laysanius* Jordan and Gilbert the illicium (Fig. 1, D) is exceptionally long and bears an elongated pennant of skin. The entire habitus of this fish suggests the ceratioid type and tends to support the hypothesis[2] that the ceratioids were derived from sargassum-living sea-mice. At first, we may suppose, the ancestral sea-mice stayed with their seaweed even after it broke loose from shore and began to sink to lower levels but afterward they gradually became independent of the weed and began to try their luck with lure and trap, floating quietly in the middle depths and devouring the foolish creatures that

[2] William K. Gregory, *Trans. Amer. Philos. Soc.*, 23: Article II, pp. 402, 403, 1933.

responded to their "bait." Indeed a comparison of the skull structure (Gregory, 1933) indicates that here as elsewhere the sea-mice are the central types. *Lophius,* on the other hand, with its immense and depressed head, very large downwardly-directed pectorals and strong pelvics, seems to represent a side-shoot lying somewhere along the road from primitive sea-mice to the sea-bats. Among the ceratioids the big-jawed melanocetids are regarded on other grounds by Regan and Trewavas as being the most primitive.

The small nipping mouths of the parasitic male ceratioids assuredly represent a secondary reduction in size of the mouth. And no less secondary has been the loss of their illicium and the development of tooth-like structures in the skin in front of the upper and lower jaws. Equally secondary is the enlargement of their olfactory sacs and the more or less cylindrical elongation of the body.

In short, the general sequence of evolution and interrelationships of the main lines of the pediculates, as pictured in Figs. 3 and 4, may be summarized as follows:

(1) At a relatively early period (? Basal Eocene) some already specialized derivative of the primitive perch-like fishes began to lurk among the rocks and seaweeds along the shore. It must have been able at times to lie still and move cautiously and, at the right moment, to leap forward after its prey. Living in the turbulent region of tide pools, it was capable of instantaneous adjustments to the buffeting waves. At this period it may have been not unlike a goby in general appearance and must likewise have had large pectoral fins with a vertically extended base. It had a long continuous dorsal fin in which the spinous portion had begun to extend forward on the top of the head. It may have been closely related to the toadfishes but had higher anterior dorsal rays, longer bony rods supporting its pectoral fins and some minor technical differences in the skeleton.

(2) A stage of close dependence upon the sargassum weed with increasingly cryptic coloration led to the

mouse-fishes (*Antennarius* and related genera). By this time the mouth-and-throat cavity had been greatly expanded, the gill-cover had grown backward over the shoulder-blade, and the respiratory cleft had traveled backward to a point behind and beneath the axilla of the pectoral fin; the base of the pectoral fin had become elongated distally, the fin had become more or less paw-like and capable of being twisted either downward to act as a foot or upward to serve almost as a hand. The first dorsal ray had become transformed into a lure in the manner described above (p. 196).

(3) Meanwhile a closely related division of primitive pediculates, the ancestral lophioids, were content merely to flatten themselves on the bottom among the seaweed, clinging perhaps to rocks and tending to creep offshore below the violence of the surface water. The head became extremely wide and depressed, especially across the rapidly widening jaws; the pectoral fins became enlarged and the body as a whole was much flattened dorso-ventrally. The elongated first dorsal ray with its small bits of skin near the top served as a bait.

(4) The genus *Chaunax,* which is intermediate in general form between the sea-mice and the sea-bats, indicates that the latter must have been derived from the former. The subopercula, becoming enormously enlarged and widened, forms the lateral margins of the resulting disk-like body. The large pectoral and pelvic fins come to resemble flippers. The lure is small or vestigial.

(5) Some of the sea-mice stay in the drifting weed until it is far out to sea and perhaps sink with it to considerable depth. *Antennarius laysanius* shows us how the bit of skin on the tip of the illicium lengthens into a pennant and suggests that this almost ceratioid form is learning how to drift free of the weed and beginning to practise fishing in the open. In extreme stages of the deep-sea anglers (ceratioids) even the pelvic bones have been lost, the pectorals are small or weak and the fish has become practically an animated bag with a more or less elaborate bait and jaw-trap.

(6) The parasitic male ceratioids have become excessively specialized, the mouth secondarily reduced in size and provided with a nipping apparatus made out of thorny projections on the rostrum and tip of the lower jaw. The more or less globose-to-cylindrical body-form is assuredly secondary and so is the great enlargement of the "olfactory" organs, the lack of an illicium, the hypertrophy of the testes and the reduction of the other viscera. Small free-swimming males which have not yet assumed such extreme specializations are recorded in several families of ceratioids by Regan and Trewavas. These authors close their account of sexual dimorphism in the ceratioids with the following interesting passages:

> Some males, except that they have no illicium, scarcely differ from the females, whereas others may differ from them in various ways. In some families the males become sexually mature as free-swimming fishes, in others they do not become mature until they have attached themselves to the females and have become parasitic on them.
>
> As an illicium, or line and lure, is characteristic of the Angler-fishes, it is evident that it is the female Ceratioids that most nearly retain the structure and habits of the original members of the group. But in the darkness of the ocean depths, if the fishes of both sexes floated about alone, attracting prey by means of a lure, it would be difficult for them to find one another. It is the males who have changed, have lost the lure, have adopted more active habits, and seek the females.

In conclusion, every change in the inferred evolution of the pediculates as summarized in the foregoing pages illustrates the principle of evolution by polyisomerism and anisomerism.[3] For example, the dorsal fin in primitive fishes is supported by a series of similar bony rods, which come well within the definition of *polyisomeres* or serially homologous structures. In the pediculates the first three of these rods become separated from the fin and then one of them, by a process of *anisomerism,* or unequalization, becomes dominant over the others and is known as the illicium or lure. In certain ceratioids the skin at the end of the illicium puts forth many outgrowths (polyisomeres) and of these in some genera certain ones

[3] Gregory, *Proc. Nat. Acad. Sci.*, 20: 1, 1–9, 1934.

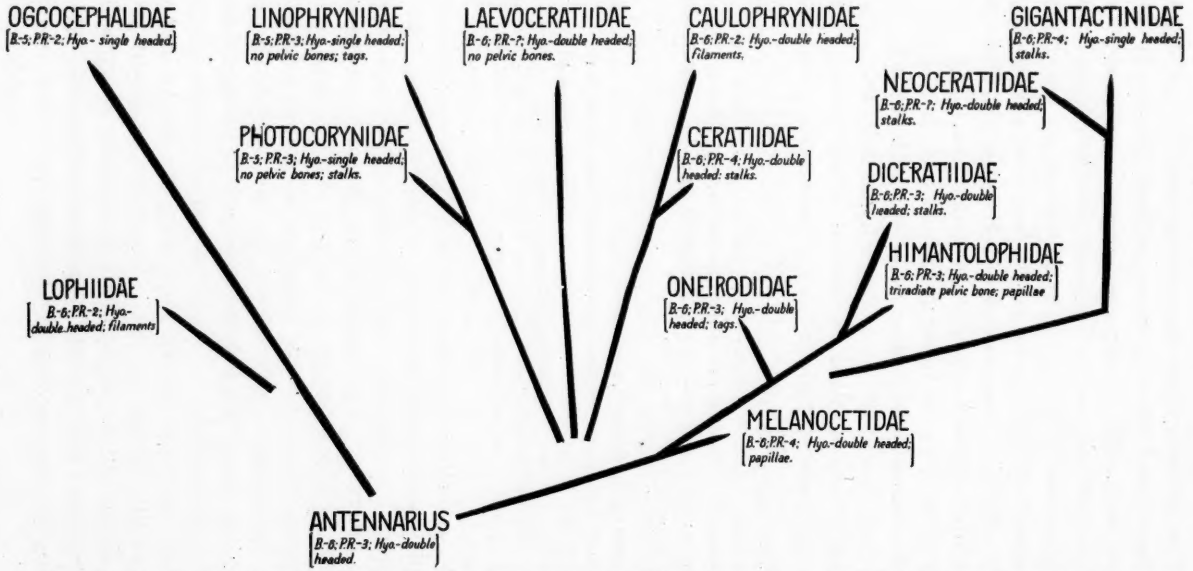

FIG. 5. Distribution of certain diagnostic characters among the pediculate families. Data chiefly from Regan and Trewavas, supplemented from other authors. B = branchiostegals; PR = pectoral radials; Hyo = hyomandibular. Stalks, papillae, etc. = lateral line organs.

are selected for either positive or negative development (anisomerism).

In the evolution of the pectoral fins the basal rods, originally four small and subequal polyisomeres, become subject to anisomerism, involving unequal enlargement of the so-called "ulna" and suppression of all but one of the others. Then in certain highly specialized ceratioids with reduced pectoral fins secondary polyisomerism is manifested in the appearance of four almost subequal rods.

Unequal emphasis of particular parts in various ceratioids results in extreme differences in the size and direction of the mouth (see p. 197). It is evident also that the very small mouths of parasitic males are by no means due to a retention from primitive fishes but to a secondary reduction.

In short, the student of piscine morphology, faced with numerous evidences of astonishing transformations of proportions, will be sceptical of certain current applications of Dollo's law of "the irreversibility of evolution."

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SIZE INHERITANCE IN MICE[1]

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BEFORE taking up the results of some mouse crosses to be described, it might be well to explain how we came to undertake them and what was their purpose.

When Mendel's law was rediscovered in 1900, I was engaged in certain breeding experiments with mice and guinea pigs, and was able to observe in my own experiments a verification of the law as regards the inheritance of albinism and other coat characters.[2] It soon became clear that Mendel's law explained satisfactorily the inheritance of color characters and structural characters of the coat in mammals, no less than that of numerous flower, foliage, and seed characters of plants. But our attention was, from the very start, attracted by a group of seemingly non-conformable cases affecting the inheritance of vitally important characters such as size of the body or of its parts. In regard to these, the Mendelian principles of dominance and segregation apparently did not hold, but the inheritance was intermediate or blending. I have been trying, for the last thirty years, to find an adequate explanation of blending inheritance and am only beginning to be satisfied with the result.

Our first study was made on the inheritance of body size and ear length in rabbits. A cross was made between the large-bodied and long-eared breed of so-called lop-eared rabbits and ordinary races which are much smaller in body size and ear length. The hybrids were intermediate between their respective parents in both regards and transmitted this intermediate condition to their offspring with only slightly increased variability.

This result, without attempted explanation, was pub-

[1] An address delivered before the Biological Colloquium at Harvard University, February 21, 1936.

[2] *Proc. Am. Acad. Arts and Sci.*, 38, 1903.

lished in 1909.[3] Shortly thereafter the Swiss zoologist, A. Lang,[4] applied to our data the interpretation which had been formulated by the Swedish botanist Nilsson-Ehle,[5] to explain peculiar inheritance ratios observed in variety crosses of wheat and oats. This is known as the multiple factor hypothesis. In the light of the subsequently established chromosome theory of inheritance, it may now be stated thus. The same genetic factor (or gene) may be represented in two or three (or more) different chromosomes. A gene for red seed coat in wheat, for example, if present in one chromosome only, gives a 3:1 ratio in crosses with white seed coat. If red is present in two chromosomes, a dihybrid inheritance ratio results, 15 red: 1 white. And if a red gene is present in three chromosomes, a 63:1 ratio results. It was also observed that 2 or 3 red genes produce a darker red than one, thus bridging the gap between qualitative and quantitative characters. We now know that wheat is polyploid and so can understand how the same red gene might be represented in more than a single chromosome. But our rabbits are not polyploid, and so the applicability of the Nilsson-Ehle hypothesis to ear length and body size is not at once obvious. It has to be interpreted this way. If the genes which influence body size are numerous and are borne in many different chromosomes, and (unlike the red seed coat character of Nilsson-Ehle's wheats) are *not* characterized by dominance, then a cross between parents differing in size will produce intermediates. Subsequent generations will continue to be intermediate in character but will be somewhat more variable. These are exactly the observed experimental results, so there has always been a presumption in favor of the multiple factor interpretation as formulated by Lang to explain blending inheritance in animals. But proof of its correctness has been difficult to obtain. Repeated crosses have been made in my laboratory between races of very large and very small rabbits,

[3] W. E. Castle, H. E. Walter, R. C. Mullenix and S. Cobb, *Carnegie Inst. Wash. Publ.*, No. 114, 1909.

[4] *Zeit. f. ind. Abst. Vererb.*, 4, 1910.

[5] Lund's Univ. Arsskrift, 1909.

first by MacDowell[6] and later by myself. I finally secured races one of which was about four times as heavy in adult body weight as the other, and which differed in coat color by four independent factors, *viz.*, agouti, extension, dilution, and English pattern. It was thought that if genes influencing size were borne in one or more of these four chromosomes, this fact should be capable of demonstration by linkage, *i.e.*, by association in later generations between larger size and the color characters of the larger parent. But no such association was found, and I grew skeptical of the applicability of the multiple factor interpretation and sought a different one. A non-chromosomal or cytoplasmic basis for blending inheritance was suggested as one possibility. This finds a qualified support in the observation that in reciprocal crosses between large- and small-bodied races of rabbits the large mother has the larger-sized offspring.[7] If the inheritance of size were due to chromosomal genes only, this should not be true. If the egg cytoplasm has a share in the genetic determination of body-size, reciprocal crosses between large-bodied and small-bodied races should be dissimilar. I shall show that a difference in body size between reciprocal F_1 populations is found also in certain mouse crosses, a result accordant with Marshak's[8] findings in a different mouse cross. Further, Little[9] has shown that susceptibility to spontaneous mammary tumors in mice is a character more strongly inherited through the mother than through the father, and he regards the basis of such inheritance as non-chromosomal.

On the other hand, there can be no doubt that genes borne in chromosomes do influence body size. In mice, for example, a dwarf mutation[10] is inherited as a simple Mendelian recessive. It becomes effective through diminished secretory action of the pituitary, normal secretion

[6] *Carnegie Inst. Wash.*, Publ. No. 196, 1914; *Jour. Exp. Zool.*, 53, 1929.

[7] *Proc. Nat. Acad. Sci.*, 20, 1934.

[8] *Jour. Exp. Zool.*, 72, 1936.

[9] *Science*, 78, 1933.

[10] G. D. Snell, *Proc. Nat. Acad. Sci.*, 15, 1929. P. E. Smith and E. C. MacDowell, *Anat. Rec.*, 46, 1930.

being indispensable to normal growth. The only undecided question is whether all size differences are gene-determined, or whether there may be a combined influence of chromosomal genes and cytoplasm.

Although our search for evidence of size genes in rabbit crosses by the method of linkage had met with no success, Dr. C. V. Green,[11] in Dr. Little's laboratory at Bar Harbor, made a similar experiment with mice and demonstrated an apparent linkage between large body size and brown coat color. As a small-bodied race he employed *Mus bactrianus* from China, which carries three dominant genes, agouti, black, and intensity, replaced by recessives in the other parent race, a non-agouti dilute brown race of the house mouse about twice as large as *M. bactrianus* in adult weight. In F_2 and backcross populations, Green at first reported all three dominant genes to segregate in individuals of smaller body size, but later he abandoned the claim, as regards agouti and dilution, being satisfied of the existence of a statistically significant difference only in the case of brown individuals. He maintained that a gene for larger size was borne in the same chromosome as the gene for brown coat and reported crossing over between the two as occurring with changed frequency as the age of the parents increased.

Green's positive result, where we had obtained only negatives, led us to repeat his experiment on similar material. I sought the coöperation of a former pupil, Dr. W. H. Gates, of Louisiana State University. He crossed an inbred strain of black-and-white Japanese waltzing mouse (a supposedly domesticated derivative of *M. bactrianus*) with an inbred strain of short-eared pink-eyed dilute brown mice. The average sizes of males in the respective parent races were about 17 grams and 26 grams. The F_1 animals were black in color and nearly as large as the larger parent race (about 25 grams) and of remarkable vigor and fecundity. Dr. Gates shipped the young F_1 animals to the Bussey Institution, where they were mated reciprocally with dilute brown mice of the same race used

[11] *Jour. Exp. Zool.*, 59, 1931.

by Green in his crosses. The backcross animals fall into four color classes, black, blue (*i.e.*, dilute black), brown and dilute brown. Several recessive characters do not appear in this backcross but would be distributed equally to all four classes (as recessives), and so their presence may be disregarded. They are piebald, waltzing, short-ear and pink-eye—all of which were recovered in other backcrosses.

These backcross mice were weaned at an age of about 25 to 30 days, the sexes being separated and kept 12 in a cage and weighed monthly between the ages of 4 to 6 months. When 6 months old, they were chloroformed, and body length and tail length were measured by Sumner's method, keeping the body stretched under a pull of 20 grams. For each animal so treated, a record was made of maximum weight at or prior to 6 months of age, body length and tail length. Data were thus obtained on a backcross population of over 1200 individuals. The order of size as regards both weight and body length was the same for both sexes, *viz.*, black, blue, brown and dilute brown. For males the average magnitudes were as given in Table 1.

TABLE 1

	Wt. in grams	Ratio	Body length in mm	Ratio	Tail length in mm	Ratio
Black	28.2	100.0	95.5	100.0	78.7	100.0
Blue	28.6	101.3	95.9	100.4	80.1	101.7
Brown	29.1	103.2	96.9	101.4	79.0	100.4
D. Br.	30.0	106.3	98.4	103.0	81.7	103.8

It will be seen that dilution increases size moderately, brown increases size decidedly, and together they increase it more yet, their effects being additive. The percentage change is greater for weight (a 3-dimensional effect) than for body length (a linear dimension). The statistical significance of the differences is unquestionable. Between blacks and browns the difference in average weight is $1.22 \pm .17$ grams; between intense and dilute it is $.67 \pm .17$ grams. In body length the difference between blacks and browns is $1.96 \pm .17$ mm.; between intense and dilute it is $.95 \pm .17$ mm.

It thus appears that Green was right in his original statement that both brown and dilution emerge from this species cross in association with larger body size. As regards agouti, the case is left open, as it does not appear in this cross; but in another cross, for a description of which space is lacking, it appears to have no significant influence on size. But we have found reason to question Green's conclusion that linked size genes cause the difference. We conclude that it is the physiological action of these color genes themselves on growth which produces the difference, as will presently be indicated.

As regards the effect of brown and dilution on tail length, we have found in both sexes an unexpected and surprising result, which appears not only in this cross but also in another cross. The order of size as regards tail length is not the same as for weight and body length, but in the same groups of individuals runs black, brown, blue, and dilute brown. The combined action of brown and dilution on tail length is about the same as on body length, but it is now dilution which is responsible for the major effect, whereas brown has only a minor effect. This relation holds in both sexes for every one of four different backcrosses, that is, in 8 distinct populations. The reality of the effect is accordingly beyond doubt.

It thus appears that genes carried in the brown and the dilution chromosomes exert different effects on growth. Both increase growth generally resulting in a larger body having greater weight, and in this regard the action of brown surpasses that of dilution. They probably function throughout growth, since males which are larger than females show a greater percentage change than females in consequence of the activity of these genes. Males have grown beyond the body size of females, and the brown and dilution chromosomes have continued their differential action during this added growth.

But apart from a general action throughout growth, it appears that the dilution chromosome induces a special acceleration of growth in the tail region, just as the short-ear gene produces a special inhibition of growth in the ear region, though it has more general effects, as we shall see.

Besides backcrossing our F_1 mice with Little's dilute brown race, we also backcrossed them to the actual *musculus* parent strain, the short-eared, pink-eyed, dilute brown race of Gates. From this backcross we obtained some 2,000 individuals. This cross allowed two additional recessive characters to put in an appearance, *viz.*, short-ears and pink-eyes. But as the short-ear gene is carried in the same chromosome as dilution and is very closely linked with it (crossovers occurring less than once in a thousand cases), the total number of phenotypes was 8, 4 being dark-eyed and 4 pink-eyed blacks, blues, browns, and dilute browns, respectively. All dilute individuals (blues and dilute browns) were short-eared, and all intense ones (blacks and browns) were normal-eared, except for the rare crossovers. The order of size among the dark-eyed was as shown in Table 2 for males (females giving a similar result). Brown, as in the backcross to Little's d br race, increases size markedly, but the previously observed plus effect of blue is more than offset by a minus effect of short-ear in this backcross. Accordingly dilute browns, instead of being larger than browns, are smaller by all three criteria of size. This shows that short-ear not only completely neutralizes the plus effect of dilution but also largely neutralizes the plus effect of brown at the same time, so that animals of the dilute brown short-eared class are only about one per cent. larger than black individuals.

TABLE 2

	Weight	Ratio	Body	Ratio	Tail	Ratio
Black	28.11	100.0	94.32	100.0	80.72	100.0
Blue se	27.81	98.9	94.36	100.0	80.75	100.0
Brown	30.31	107.8	96.41	102.2	82.74	102.4
D. Br. se	28.46	101.4	95.33	101.0	81.06	100.5

We need not go into a description of the pink-eyed classes. In general, they average slightly smaller in body size than the corresponding dark-eyed classes, although the pink-eye gene was derived from the larger-bodied parent race; and so, on a linkage hypothesis, we should have expected an opposite effect.

The question now arises, are we dealing with the effects of size genes borne in the same chromosomes as the color genes (Green's assumption), or are we dealing with physiological effects on growth of the color genes themselves and of the short-ear gene?

It will be freely admitted, I think, that the short-ear gene, when homozygous, has a retarding effect on growth not only of the ears but, to a lesser extent, of the entire body. This was the conclusion reached by Snell. Yet when present as a recessive in heterozygotes, it has no apparent influence. This must have been its condition in substantially all the blue individuals produced by the backcross to Little's d br race, yet those individuals were larger by all three criteria of size than the blacks, their sibs, which were free from short-ear even in a heterozygous state.

Blue, we must assume, either itself has an opposite (accelerating) action on growth, or is closely linked with a gene which has such action (Green's hypothesis). If the accelerating action were due to a third gene linked with dilution, that action should become effective when *either* dilution or short-ear became homozygous, since, if the third gene is closely linked with dilution, it must be closely linked also with short-ear. But in reality we find that when blue is homozygous and short-ear heterozygous, growth is accelerated. If, however, short-ear as well as blue be homozygous, then growth is inhibited. The inhibiting action of short-ear on growth more than offsets the accelerating action of blue. No third gene need be hypothecated.[12]

At last we are beginning to get our hands on size genes, the thing we have been looking for all these years. By their physiological action on growth, brown and dilution are genetic accelerators; short-ear and, in lesser degree, pink-eye are inhibitors of growth. Though all four were introduced into the same cross from the larger parent, and thus on a linkage theory should emerge in a backcross in association with larger size, we find that two of them do

[12] See *Genetics*, 21, 1936, for further details.

and two of them do not emerge in such association. In other words, the linkage theory is not substantiated.

We are now in a position to formulate a multiple factor theory of size inheritance more nearly adequate than we could formulate twenty-five years ago. There undoubtedly are numerous genes borne in chromosomes which exercise an influence on growth. Genes which have been discovered by reason of other and seemingly quite different influences which they exert, also exert an influence on growth of the body as a whole. But these influences are not equivalent in amount one with another, nor are they all positive in action. Some are accelerators, others inhibitors. Some accelerate a little, others much. Some retard a little, others much. It is useless to try, as I once did, to devise a formula which would tell us how many gene differences are involved in a size cross. G. H. Shull at the time pointed out the fatal defect in such a formula, its basic assumption that all size gene effects are of like magnitude or in one direction. This they are not, as our mouse crosses show.

On the other hand it is possible that non-chromosomal structures may share in the genetic determination of size, though such an idea is considered heterodox by advocates of the gene theory. In reciprocal crosses between large-bodied and small-bodied races of rabbits, mothers of the larger race produce offspring significantly larger, as already stated. The same is true in two reciprocal backcrosses of mice, one being that already described, the other a backcross of F_1 animals from a *bactrianus* cross. The large race mother in each case produced larger-bodied offspring than the F_1 mother, as regards both weight and body length. It remains to be shown whether this is a genetic influence carried in the egg of large race mothers, or a hormonal influence exerted by the glands of the mother on embryos during gestation. But on theoretical grounds I, for one, am inclined to think that we may have gone too far in ascribing heredity exclusively to the action of genes borne in the chromosomes.

A TECHNIQUE OF TRANSPLANTATION FOR DROSOPHILA

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INTRODUCTION

THE transplantation method has been applied to the study of several larger insects, among them Celerio (Bytinski-Salz, 1933) and Ephestia (Caspari, 1933), but has not previously been made use of in studies of Drosophila species, presumably because of the small size of these organisms. The advantages of utilizing transplantation methods in studies of development in organisms as well known from the genetic standpoint as are several of the Drosophila species, are so obvious that they scarcely need be stated. It is the purpose of the present paper to describe a technique of implantation which we have successfully used in the investigation of certain problems concerned with development in *Drosophila melanogaster* (Ephrussi and Beadle, 1935; Beadle and Ephrussi, 1935). It should be pointed out that this technique does not permit of the fixation of an implant in a predetermined position in the organism. Often the implants do become fixed and may, in the case of implanted ovaries, become fixed in the normal position for these organs.

The essential part of the technique described here is the actual operation of injection of the desired tissue by means of a micro-pipette. In the development of a method of doing this, we were aided by the brief description given by Stark (1918) of the method used by her in injecting masses of tumor tissue into the body cavities of Drosophila larvae. In addition to describing in some detail this essential operation, we shall describe briefly various accessory methods concerned with growing and handling material, several of which have previously been

used by other workers. Many of the steps of the technique of the injection are empirical and can, no doubt, be improved.

We have used the technique in implanting gonads and various imaginal disks. It is obvious that it can be used also for the implantation of other organs and tissues, for the injection of liquid substances, etc. In such special cases, certain modifications may be necessary.

Collection of Eggs

We have used two simple methods of collecting eggs, one designed for single females, the other for larger numbers. The first involves the use of shallow metal food containers made of non-corrosive sheet-metal such as nickel, of a thickness of about 0.2 mm. These boxes are of the dimensions $40 \times 15 \times 3$ mm and have a handle projecting about 30 mm at one end. They can be used conveniently in standard shell vials of the dimensions 100×25 mm. For larger numbers of eggs collected from many females, we use as food containers Petri dishes about 50 mm in diameter and about 15 mm deep. These are placed, without covers, in straight-sided glass vessels about 80 mm in diameter and 130 mm deep which are kept in a horizontal position and closed with a cotton stopper enclosed in cheese-cloth. The principal advantage of this method is that flies from stock cultures can be placed in the egg-collecting vessels directly without etherization, which retards egg laying. As a medium on which eggs are laid, the standard cornmeal-agar formula (Bridges and Darby, 1933) is used. Animal charcoal may be added to increase the contrast between eggs or larvae and food. The food containers are filled level-full, allowed to harden, and the surface painted with a rather heavy suspension of fresh yeast. The females are allowed to deposit eggs for the desired period of time, the containers removed, the eggs allowed to hatch and the larvae transferred to appropriate containers as described below.

CULTURE OF LARVAE

For growing larvae, a shallow container has the advantages of having a large food area per unit volume and of permitting the removal of larvae with ease. Petri dishes can be used, but have the serious disadvantage of not allowing sufficiently rapid diffusion of gases. The larvae often suffocate if the cover is sealed by moisture or the growth of yeast is too rapid. We use heavy glass dishes of two sizes, one about 90 mm in diameter and 50 to 60 mm deep, the other about 125 mm in diameter and 60 to 70 mm deep. These have vertical walls with an external rim at the top. They are closed with a square of cloth held on by a heavy rubber band. A layer of food about 20 mm in thickness is poured into these and, after cooling, painted with a heavy suspension of yeast. The smaller size provides for good growth of from 50 to 100 larvae, the larger for from 100 to 200. Known numbers of young larvae can be quickly transferred to these dishes with a small spatula made from a heavy dissecting needle.

For obtaining larvae of accurately known stages of development, it is better to take larvae hatching within a given time interval than to take those from eggs laid within a similar time interval. This insures the elimination of eggs laid in an advanced stage of development and tends toward the selection of larvae of uniform rates of development. Kerkis (1931) has used this system and the method of collecting pupae described in the following paragraph.

If organs from pupae of known stages of development are wanted, the time of pupation serves as a convenient index. Larvae can be allowed to pupate on the walls of the culture dishes, and removed at desired intervals to moist filter paper in vials. During the formation of the puparium, the larvae assume a characteristic barrel shape. For a period of about one hour (25° C.) they remain "white" and thereafter begin to darken. This darkening can be used to advantage in selecting individuals which have started puparium formation within the previous hour.

Equipment and Procedure

In the procedure described here, two persons work together, one dissecting out the desired organ and making the actual injections, the other preparing the host larvae and holding them during the injection. It is possible for one person to carry out the entire procedure, but the two-person arrangement has the advantages of decreasing the time required for a given series of injections, which is often important, and of permitting a change in relative rôles of the two workers. This latter point is of considerable importance because of the fact that continuous dissection and injection is very fatiguing.

Apparatus: Two binocular microscopes are arranged so that they can be used from opposite sides of a rather narrow table. One, used by person A, is equipped with a complete stand and has a mechanism for the rapid change of objectives. The other, used by B, is a body tube mounted on an adjustable stand. It is arranged to face the first and adjusted at an angle of about 45° so that the centers of the fields of the two coincide at stage level. To the left of microscope A, is fastened in a convenient position a hypodermic syringe which leads by means of a flexible metal capillary tube to a pipette-holder located to the right of microscope A. The assembly that we use is that of the standard Chambers' micro-injection apparatus.

The micro-pipette plays a very important rôle in the operation of injection and is the item of equipment with which we have had most difficulty. It is made by drawing out with a micro-burner a glass capillary with an external diameter of about 0.7 mm and a wall thickness of about 0.1 mm, to a finer capillary shaft of an external diameter of from 0.1 to 0.16 mm, this depending of course on the use to which it is to be put. The bore of the shaft should be from 0.06 to 0.12 mm in diameter. The shaft is broken so that its length is 2 or 3 mm. At the base of the shaft a constriction in the bore is made by careful heating, point down, with a horizontal micro-flame. For ordinary use,

the internal diameter of the constricted portion of the shaft should be of the order of 0.01 to 0.03 mm. This constriction in the bore should be as abrupt as it is possible to make it. Otherwise the tissue tends to become wedged into it and injured as it is drawn into the pipette. The constriction has two important functions, (1) to block the disk or other piece of tissue in the shaft as it enters the pipette and thus prevent its entering the larger shank, where it is both difficult to observe and control, and (2) to act as resistance to the flow of liquid through the pipette, which greatly facilitates the regulation of the amount of injected liquid. The last step in the making of a satisfactory pipette is the preparation of a good point. The shaft should be broken at an angle of 30° to 45° to its axis in such a way that the extreme tip is very sharp (Fig. 1). Such a point can be made by careful chipping under a binocular microscope with a dissecting needle or by careful grinding on a fine-grained hone. A needle of satisfactory dimensions for optic imaginal disks of mature larvae is diagrammed in Fig. 1. The exact dimensions

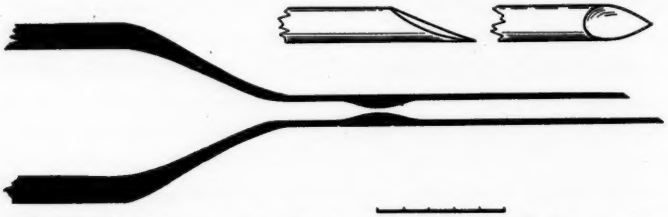

FIG. 1. Diagram of a micro-pipette suitable for the injection of optic disks from mature larvae. Drawn to scale except that the shaft is somewhat shortened. Two views of a desirable type of point shown above. Size indicated by scale below, each division of which represents 0.1 mm.

are of course governed by the size of the organ or piece of tissue to be injected; for example, for wing disks it should be somewhat larger, for larval ovaries, somewhat smaller.

The dissection: Preparatory to dissection, the larvae or pupae are washed for 10 to 30 seconds in 95 per cent. alcohol, then rinsed in a Ringer's solution (NaCl, 7.5 gr.;

KCl, 0.35 gr.; and $CaCl_2$, 0.21 gr. per L. H_2O). Dissection is made in a drop of the above Ringer's with sharp steel needles. The exact procedure of dissection varies according to stage of development and to the organ or tissue desired. The monograph of Strasburger (1935) describes briefly and figures the internal structure of *D. melanogaster* at various stages of development. The sex of larvae is readily determined by external examination as described by Kerkis (1931).

Preparation of host larvae: While person A makes one or several dissections, B prepares the host larvae by placing them on a slide, rinsing them with a drop of 95 per cent. alcohol, drying them with filter paper and placing them in an appropriate vessel for etherization. Etherization should be long enough to completely anesthetize the larvae and at such a rate that they become extended. After etherization, the larvae are arranged on the slide in convenient positions for injection, usually at right angles to the axis of the slide.

Injection: The injection is made, usually under relatively low magnification, in the following way: The pipette-holder is taken in the right hand and the syringe manipulated with the left. The syringe and metal capillary tube having previously been filled with Ringer's solution (not continuous through the needle holder to the pipette), a small amount of Ringer's is drawn into the pipette. The tissue to be injected is then drawn slowly into the shaft of the pipette. In the case of imaginal disks of mature larvae, the cross-sectional area of the disk is usually greater than the cross-sectional area of the bore of the shaft of the pipette. Consequently considerable pressure may be needed to draw the disk into the pipette. The elasticity of the disk is usually sufficiently great to allow considerable attenuation without injury, as is clearly shown by the fact that it can develop normally after injection. During this process the first function mentioned above, of the constriction in the shaft of the pipette, becomes obvious. The pipette is now lifted away from the stage and B places a larva on the stage in posi-

tion for injection. The larva is held in position with a rather heavy, blunt dissecting needle, the end of which is bent at a slight angle. This needle is held approximately parallel to the larva and the holding is done largely by surface adhesion and with very little pressure on the larva. The pipette is inserted on the side or ventral surface of the larva, usually nearer to and toward the posterior end. Other positions during the injection are possible and may be desirable in certain cases. The tissue in the pipette together with a small amount of saline is injected and the pipette withdrawn.

The above description of the actual procedure of injection is necessarily inadequate. The precise way of holding the host larva, the manner of inserting the pipette, the speed at which the tissue and saline should be injected and the rate and direction of withdrawal of the pipette and its relation to the rate of flow of liquid are all matters that can best be learned by experience. The most frequent difficulty and the hardest to learn to overcome is "blowing out" of the larva, *i.e.*, flowing out of part of the internal organs. When everything is working properly, little difficulty from this source is met with.

Treatment of injected larvae: The procedure to be followed after injection will vary according to the stage of development of the host larvae. Most of our work has been done with hosts almost ready to pupate. These are merely transferred with a small spatula to sterile moist filter paper (about four thicknesses) in shell vials. These, stoppered with a rather tight cotton plug, are placed at the desired temperature for further development. Usually sufficient moisture is retained in these tubes until all successfully operated individuals have emerged as adults. In the case of younger larvae, food must be provided. In our experience, which in this case is rather limited, it is not necessary to use bacteria-free medium and yeast. Larvae may be conveniently provided with food in the small metal boxes described above in connection with the collection of eggs.

Efficiency of the Technique

Naturally the speed and efficiency of the technique described above vary with the type of transplants that are made. With optic disks implanted into larvae shortly before pupation and with conditions generally favorable, about 30 injections per hour can be made. The percentage of such injected individuals which reach maturity varies rather widely, averaging for several thousand individuals about 30 per cent. and reaching a maximum for favorable material and "good" days of about 60 per cent. Thus on one day, 163 injections were made and from these 102 adult flies were obtained. In the case of "standard" optic disk implants, 80 to 90 per cent. of the emerged individuals have differentiated supplementary eyes. In individual series, this percentage often reaches 100. Transplantations of larval ovaries have been made with an efficiency not far different from that observed in transplantations of optic disks.

Summary

A technique of transplantation, applicable to Drosophila species, which consists of injection by means of a micro-pipette, is described in some detail.

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CHROMOSOME STRUCTURE IN TRADESCANTIAE VII[1]

FURTHER OBSERVATIONS ON THE DIRECTION OF COILING IN *TRADESCANTIA REFLEXA* RAF.

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THE individual chromosomes of liliaceous plants at first metaphase when prepared by certain methods consist of pairs of large regular coils, each coil of which is a chromatid. The chromatids can be observed easily enough to follow their path in detail. The mode of crossing-over as related to the phenomenon of coiling is discussed in a separate paper (Nebel and Ruttle, 1935). The chromatids, which are extremely obvious at first metaphase, can with additional care be traced through the successive divisions, and their history has been summarized recently (Nebel, 1935). In the present paper the direction of coiling at first metaphase will be discussed as a separate phenomenon. This study should serve as a basis from which the significance of related phenomena may be approached.

In *Tradescantia reflexa* the major meiotic coil commences to form immediately after pachytene. It has not been possible to make preparations showing details of the coils as well as details of the entire chromosomes until after diakinesis. During diplotene and early diakinesis the chromatids are thick and not as distinct as during first metaphase. At first metaphase there is a minor coil actually perhaps merely a minor undulation or wave visible in the major coil (Kuwada, 1932), which is for the present disregarded.

Recent papers on the direction of coiling of the major coil at first division in liliaceous plants have shown that

[1] Approved as Journal Paper No. 59 May 30, 1935, N. Y. State Agricultural Experiment Station.

this phenomenon apparently does not conform to a simple scheme. Sax and Humphrey (1934), working on an undescribed species related to *T. reflexa,* found that the two chromatids on the same side of the main opening (synaptic plane) previous to first metaphase always coil in parallel. The direction of the major coil is at random for the homologous chromosomes of each tetrad. "Within each homologue the direction of coiling may change at the fiber constriction but there is a tendency for the coiling to be in the same direction on both sides of the fiber. The direction of coiling may also change at an interstitial chiasma." Sax (1935) found practically the same to be true in *Rhoeo discolor.*

Iwata (1935) concludes that perhaps even each chromatid in *Lilium* is coiled independently of the others. No law according to which coiling is regulated was found. Huskins and Smith (1935) apparently find modified random coiling in *Trillium.* Chromatids on the same side of the main opening spiral in parallel, excepting for the exchanges at chiasmata. Paired chromosomes commonly coil in opposite directions; in paired ends all four chromatids often coil in the same direction. Shinke (1934), who has made extensive studies on *Sagittaria,* does not discuss the direction of coiling in individual chromosomes.

Nebel (1932 *a*) considers that there is a significant deviation from randomness of coiling in *Zebrina pendula* and *T. reflexa* and (*idem* 1932 *b*) that there exists a condition tending towards constancy of direction of coiling in a given chromosome in *Rhoeo.*

These observations in general agree that coiling is at random, except for the modifications stated above. To date no statistical analysis of the direction of coiling in definite parts of the gene string has been made, aside from the work of Nebel (1932 *b*) on *Rhoeo* and that of Sax (1935) on the same plant. Nebel (*loc. cit.*) suggested a relative constancy of direction of coiling, at least in certain parts of the *Rhoeo* ring, while Sax considered the direction of coiling to be at random for any specific chromosome in the ring.

MATERIALS AND METHODS

A clone was used from an x-rayed plant of *T. reflexa* (C.P.H. 7) which showed at meiosis three rings or chains of two, four and six chromosomes, respectively. After smearing the sporocytes the preparations were immediately placed for 30 seconds in 60 cc. Ringer solution made up as follows: 0.9 g. NaCl; 0.01 g. KCl; 0.01 g. $CaCl_2$; 0.002 g. $NaHCO_3$ in 99 cc. distilled water and diluted one half. After this pretreatment the preparations were fixed in 2 per cent. osmic acid dissolved in 1 per cent. platinum chloride, dehydrated, and stained with crystal violet.

OBSERVATIONS AND DISCUSSION

T. reflexa has 6 haploid chromosomes of approximately equal length with submedian kinetochores. (Kinetochore is a convenient term for the spindle fiber insertion region; see Sharp, 1934, p. 116). In plant C.P.H. 7 it is assumed that three reciprocal translocations between non-homologous chromosomes account for the catenation. The catenations allow one to pick out corresponding groups of chromosomes in different sporocytes, even though the individual chromosomes in the respective groups could not be identified.

Primary attention was given to the direction of coiling in the ring or chain of two chromosomes of clone C.P.H. 7. Since both homologues in this group have submedian kinetochores and no individual markings, a record of the direction of coiling in parts of this pair does not represent a record of the direction of coiling in definite parts of the gene string. In this set of observations change in the direction of coiling was recorded only as occurring at the kinetochore. Changes in direction of coiling in interstitial parts apparently due to chiasmatization as described by Huskins and Smith (1935) have also been seen in *T. reflexa* (see Nebel and Ruttle, in press).

The single pair was observed in 40 different cells from 8 slides made from 4 different anthers of 2 different

TABLE 1

FREQUENCIES OF COILING-PATTERNS OBSERVED IN A DISTINCT GROUP OF TWO CHROMOSOMES FROM AN X-RAYED PLANT OF *T. reflexa*, C.P.H. 7

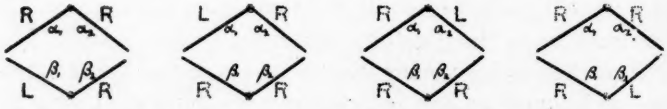
Slide number	Direction of coiling					
	RR LR	LL RL	LL LL	RR LL	RL RL	RR RR
7/27/34–7	5		1	2		1
4/9/35–1		6	1		2	
4/9/35–4		1			1	
4/12/35–1		2				
4/12/35–3	2					
4/12/35–4	1					
4/12/35–6	1					
4/12/35–8	6	5	2	1		
	15	14	4	3	3	1

plants of the same clone (see Table 1). Each smear comprised the contents of one half-anther only (2 loculi), excepting smear 4/12/35–8, which contained two anthers from the same bud.

Since both chromatids of a chromosome commonly spiral in the same direction R and L are used to designate the direction of coiling between the chromosome ends and the kinetochores, in this case, then, R or L designate the direction of coiling in two sister chromatids. A univalent can thus be fully described by the formula LL, RR or RL, which means that the two arms of the chromosome in question are both sinistrorse, both dextrorse or one sinistrorse and the other dextrorse, respectively. In the case of a tetrad four letters are used, each one designating the direction of coiling in one arm of the two homologues. The designation $\frac{\text{RR}}{\text{LR}}$ for the tetrad coiling pattern in Table 1 is the equivalent of the designation RR LR in the text. The former arbitrarily assumes that the two chromosomes are lying horizontally one above the other, although actual observations on homologues not so arranged are included.

Table 1 shows that the patterns RR LR and LL RL account for 72 per cent. of the observations. Type RR LR was apparently more prevalent in some anthers, type LL RL in others. In slide 4/12/35–8 comprising two anthers both types were found on the same slide. All other patterns to be expected under randomness were found, but their frequency was depressed.

It is concluded that in the group of two chromosomes under observation there are two distinct patterns of coiling which tend to re-occur with a frequency considerably exceeding random expectation. The frequent occurrence of any particular pattern such as RR LR does not necessarily indicate that the direction of coiling is constant in any specific part of the gene string, but that there is some mechanism which tends to produce a relative constancy of sequence in the alternative directions of coiling, here called a pattern. For illustration any two homologous chromosomes with submedian constrictions, such as those in Table 1, each have practically indistinguishable arms α_1 α_2, β_1 β_2, respectively. Since neither the chromosomes nor their arms are distinguishable there are four possible ways in which the arms could be differently coiled and yet produce the pattern RR LR thus:



Patterning in this sense would also appear to fit the observations made on the rings or chains of four and six chromosomes from the same clone. Here again the chromosomes carry no markers, so that successive observations of coiling on the same ring or chain do not necessarily represent successive observations on identical parts of the gene string. The best available figures were recorded first and additional data were added with the intention to fit the original scheme. This may be illustrated from 45 observations of individual chromosome

parts in the ring or chain of four chromosomes from seven cells in smear 7/27/34.

Thus in the seven cells observed the order of coiling in the ring or chain of 4 successive chromosomes in

cell 1 is	RL	RR	LR	LL	Complete observation of 1 ring
" 2"	—	—	LR	LL	
" 3"	RR	RR	L–	–R	
" 4"	R–	R–	–R	LL	The direction of coiling in the
" 5"	–L	LR	—	LL	parts indicated by the —sign
" 6"	R–	RL	LR	LL	could not be determined.
" 7"	RR	R–	–R	LL	

Taking the most prevalent direction of coiling in each successive part the pattern becomes R? RR LR LL.

A series of 28 similar observations on the same ring made from a single half-anther dated 9/19/34 gave a slightly different pattern, which was LL LR LR LL.

From slide 7/27/34, 63 observations were made on the ring or chain of six chromosomes. Taking the most prevalent direction of coiling in each successive part as stated for the ring or chain of four the pattern obtained was LL RR LR LL LR LL. Similarly 51 observations from slide 9/19/34 gave the pattern RR RR RR LL LR LL.

When the pattern R? RR LR LL of the ring or chain of four from slide 7/27/34 is taken together with the pattern LL RR LR LL LR LL of the ring or chain of six from the same slide the ratio of dextrorse to sinistrorse units is 8 R (plus one questionable R) : 11 L. Similarly, when the pattern LL LR LR LL of 9/19/34 is taken together with the pattern RR RR RR LL LR LL of the same date, the ratio of dextrorse to sinistrorse units is likewise 9 R : 11 L.

As in the observations on the group of two chromosomes the observations on the rings or chains of four and six chromosomes suggest that the fitted patterns do not correspond to any constancy in the direction of coiling in a given region of the gene string. Both groups of observations, however, suggest that there appears to be

within the nucleus some factor besides randomness governing the direction of coiling. This factor apparently varies between anthers.

It may be incidental that in the present observations two patterns of coiling were found in each case which seem to overlap in parts. These can be written as follows:

For the group of two:	(a)	RR	*LR*				
	(b)	LL	*LR*				
For the group of four:	(a)	R?	RR	*LR*	*LL*		
	(b)	LL	LR	*LR*	*LL*		
For the group of six:	(a)	LL	*RR*	LR	*LL*	*LR*	*LL*
	(b)	RR	*RR*	RR	*LL*	*LR*	*LL*

This may indicate that certain parts of the pattern have a higher degree of stability in different half-anthers than in others. On the other hand, a more extensive search possibly might exhibit more than two patterns for each configuration in a clone, such as C.P.H. 7. A certain amount of overlapping in various regions of such patterns would be expected even if the patterns originated at random.

In view of the findings on *T. reflexa,* C.P.H. 7, earlier work on *Rhoeo* (Nebel, partly unpublished, summarized 1932c) can be interpreted as a pattern conformation, in which the 158 observations on the direction of coiling in individual arms of chromosomes from 13 sporocytes from 3 different plants conform to the pattern,

LL LR RL R? RR RL LL RL LR RL RR LR

This pattern was more variable in certain regions of the chain than in others, which also agrees with the findings in *T. reflexa.*

As an explanation for the phenomenon of patterning McClintock verbally suggested an effect of position of the chromosomes in the nucleus as a possibility. Within the massive nuclei of *T. reflexa* it seems tempting to imagine that successive nuclear generations may have

similarly arranged chromosomes, which in turn might give rise to similar direction of coiling.

The direction of coiling is as yet a phenomenon not fully understood. Disagreement in the findings of various workers examining different plants or even the same plants may be due to variability within a plant. As an example of such variation the following compilation of various data may be of value:

In *T. reflexa,* C.P.H. 7, the observations on the group of two chromosomes in Table 1 show non-reversal of coiling at the kinetochore as against reversal in the ratio 100:78. Comparable observations on rod-shaped gemini from normal material give the ratio 100:38. Earlier data (Nebel, 1932 *a*), which included observations on both rod and ring-shaped gemini, give the ratio of 100:73. The data of Sax and Humphrey (1934) on coiling in pairs of chromosomes from a normal species related to *T. reflexa* show non-reversal to reversal at the kinetochore in the ratio 100:42 (*loc. cit.,* p. 359) which agrees closely with the ratio 100:38 observed by us on rod-shaped gemini from normal *T. reflexa* (above).

In *T. reflexa,* C.P.H. 7, observations on the ring or chain of 6, slide 7/27/34, show non-reversal of coiling at the kinetochore as against reversal in the ratio of 52:100. It would be premature to ascribe this inverse relation directly to the effect of translocations.

In *Rhoeo,* Nebel (unpublished data summarized, 1932 *c*) finds non-reversal of coiling between terminal chiasmata in the ratio 100:87, while Sax (1935) in the same plant finds equality of either alternative. Non-reversal as against reversal of coiling at the kinetochore in *Rhoeo* was observed in the proportion 100:63 by Sax and in the proportion 100:75 by Nebel, Nebel's actual figures being 32 non-reversal to 24 reversal.

It would seem that sufficient observations from various workers have accumulated to warrant certain conclusions: The direction of coiling is apparently not a stable condition of a certain part of the gene string and can

therefore not be used as a genetic marker to identify certain chromosomes or pieces of chromosomes. Whether the formation of patterns of coiling is of general importance can not be decided until those workers who have suggested random coiling present statistical evidence of their findings. The possible bearing of observations on coiling on the problem of crossing-over is discussed in another paper. The mechanism of coiling itself is as yet completely unexplored. The existing suggestions as to the origin of the coils must be put to further test.

Kuwada (1927) has suggested that coiling of the chromonema is caused by contraction of the chromonema within the confinement of the matrix. The matrix holds the coil in place. Huskins and Smith (1935) advanced a hypothesis of heterogonic growth, which according to Sax (1935), can scarcely be made to cover the complicated conditions of the *minor* coil. Kuwada and Nakamura (1934), extending Kuwada's hypothesis, have assumed a matrix within a matrix, the inner one to hold the minor coil intact, the outer one to preserve the larger coil. This again appears to be doubtful as Nebel (1932 *a*) concluded that each chromonema has its own matrix and each chromosome (dyad) of first metaphase contains four, not two, chromonemata.

To clear these contradictions, a careful analysis of the origin of the coils is much needed. A study of the mechanism of coiling leads to those phases of interaction between the gene string and its immediate surrounding, which are at present least understood.

SUMMARY

In an x-rayed strain of *T. reflexa* showing rings or chains of two, four and six chromosomes, respectively, serial observations indicate that the direction of coiling tends to fall into certain patterns within a given half-anther. These patterns do not necessarily belong to given chromosomes or parts of chromosomes but appear to belong to a given group of chromosomes. Random

coiling is thus considered to be modified within an anther. Similarity of patterning between half-anthers is suggested, but over a long period and with a sufficient number of observations the various patterns between different anthers may form a random series.

ACKNOWLEDGEMENT

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ADDED DURING PROOF

(1) Further observations on the right-left condition of coiling of the distinct pair of chromosomes recorded for a definite clone in Table 1 of this paper were made during the fall of 1935 from 5 slides. The results were:

RR LR	LL RL	LL LL	RR LL	RL RL	RR RR
10	16	0	2	2	1

This count is identical with that obtained a year ago. It further confirms the previous conclusion that random coiling is modified by a tendency to conform with a certain pattern, or as in the present case, with two alternative patterns which reoccur at least over the period of several months.

(2) Matsuura (*Jour. Fac. Sc. Hokkaido S. V*, 3: 233–250. 1935) recently studied coiling in *Trillium kamt-schaticum* Pall. Matsuura's data show patterning in the A-chromosomes in favor of the configuration $\frac{\text{L–L}}{\text{L–L}}$ (Table I and VI) in the B-chromosome in favor of $\frac{\text{R–R}}{\text{R–R}}$ (Table III) and in the D-chromosome in favor of $\frac{\text{–L}}{\text{–L}}$ (Table III and VI). Matsuura argues in favor of random coiling and considers the deviations from expectation as not significant. It is hard to see why in Matsuura's data the deviations from expectation should be less significant than those observa-

tions that conform. In *Trillium* patterning appears confined to 3 out of 5 bivalents and is thus weaker and less general than in *Tradescantia.*

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THE STATISTICAL ANALYSIS OF THE DISTRIBUTION OF POND MOLLUSCS IN WESTERN CANADA

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THE following account deals with the distribution of fresh-water mollusca in the western part of Canada, and consists of the results of the study of the fauna of 315 ponds and small lakes. It was considered desirable to make a more precise analysis of their molluscan fauna, from the standpoint of local and geographical distribution, than has previously been attempted with any group of invertebrate animals. The use of statistical methods has led to important advances in plant geography, and it is believed that the following pages, which are to be regarded only as a preliminary essay, will indicate that when carried out in more detail, equally interesting results will come from the application of such methods to the study of animal distribution. Nearly thirty years ago Forbes (1907) formulated a method of obtaining a measure of the association of one species with another, but unfortunately he utilized it in the study of a group of fishes, the Etheostominae, to which it was not particularly well adapted. Since Forbes's work was published the method seems to have been forgotten; at any rate very few references to it are to be found in the literature. The point of view taken here is an extension of that of Forbes.

Most data on geographical distribution are not adapted to statistical analysis. The collecting is done in a more or less haphazard fashion; collecting places are widely and unevenly scattered in space and time; and the number of observations is usually inadequate. The result is a heterogeneous mass of material which can not be handled mathematically. Effort has been specially di-

rected in the present study toward obtaining a homogeneous body of data amenable to mathematical treatment. All the collecting was done by one person.

The territory examined in the course of this investigation lies in the Provinces of Manitoba, Saskatchewan and Alberta, Canada, between 49° 30′ and 56° North Latitude. The general plan of work was to examine twenty-five or more ponds in several districts, *viz.*, Saint Vital in Manitoba, Moose Mountain, Touchwood and the Missouri Coteau in Saskatchewan, and Viking, Tofield, Cooking Lake and the Peace River Country in Alberta. In addition to these, other ponds were studied in the intervening territory. In this way it was possible to obtain a representative sample of the fauna of this type of habitat over the country as a whole. All the ponds studied are situated in the Steppe and Forest-Steppe regions of western Canada. To the local population these are known respectively as the Prairie and the Grove Belt, but it appears to be desirable in this paper to designate them by the more specific names which are applied to the comparable regions of northern Asia. These regions of western Canada occupy a flat or gently rolling type of country, generally covered with grassy vegetation, to which is added in some places small groves of aspen (*Populus tremuloides*). This is especially true along their northern limits where they border on the forested areas, but aspen also occur in a few more southern localities (*e.g.*, near Moose Mountain). Although several great rivers run through this territory the minor channels of drainage are little developed, and there are many thousands of ponds and small lakes. The explanation of their presence lies in the fact that the continental ice sheet has only recently receded from this region, and there has been insufficient time for the full development of the drainage basins.

Ten species of fresh-water gastropods are known to occur in the ponds and small lakes of this part of Canada, namely: *Lymnaea stagnalis jugularis* Say; *L. palustris*

(Müller); *L. caperata* (Say); *Planorbis trivolvis* Say; *P. exacuous* Say; *P. arcticus* Möller; *P. umbilicatellus* Cockerell; *Planorbula campestris* Dawson; *Aplexa hypnorum* (Linne); *Physa gyrina* Say.

In addition, two pelecypods, *Sphaerium occidentale* Prime and *Pisidium* sp., have been collected in the course of this work. The first-mentioned species has been found in seven ponds, and the other only once. This small number of records renders their inclusion in this discussion undesirable. The gastropod species listed are not at all uniformly distributed. Each of them must be regarded as having its own particular preferences of habitat, which never entirely coincide with those of any one of its associates. One of the objects of this paper is to call attention to some of these differences in habitat requirements and distribution.

The method of analysis adopted in this work is as follows; assuming the occurrence of any particular species in any one pond to be purely a matter of chance, it is possible by means of a simple formula to calculate the number of times any two species may be expected to be found together in the total number of ponds. The ratio between this computed value and the actual value, as observed in the field, gives a measure of the tendency of the two species to occupy similar habitats, in other words, a measure of the tendency, or lack of tendency, of the two species to associate. These calculations are facilitated by the use of symbols as follows; a being the total number of ponds examined during the whole of the investigation; b being the number of ponds in which one of the two species was found; c being the number of ponds in which the other of the two was found; and d being the number of ponds in which *both* species were found together. The likelihood of the first species being found in any one pond under conditions governed wholly by chance will be $\frac{b}{a}$, and of the second species $\frac{c}{a}$. The chance that they will both be found together in a single

pond will therefore be $\frac{b}{a} \times \frac{c}{a}$, and the number of such occurrences to be expected in the whole series of ponds examined will be $\frac{b}{a} \times \frac{c}{a} \times a$. If this computed value is divided into the actual number of occurrences in common observed in the field (d) the result is a measure of the association of the two species and may be termed the *coefficient of association.* The formula may be somewhat simplified as follows,

$$\frac{d}{\frac{b}{a} \times \frac{c}{a} \times a} = \frac{ad}{bc}$$

If the number of observed occurrences corresponds with that expected, the value of the coefficient of association is unity, and if this were obtained there would be no need to invoke any other factor than mere chance to account for the number of associations. If, however, the coefficient of association is distinctly greater than unity it clearly indicates some other factor in the nature of physiological requirements or restrictions common to the two organisms. Conversely, a correlation noticeably lower than unity must indicate an important difference in the requirements of the two species. Although it does not indicate the nature of these requirements or restrictions, it does state the physiological problem more explicitly than most other methods of approach.

The coefficients of association of the ten species of molluscs which commonly inhabit ponds in the western part of Canada are given in Table I. It is important to note that these values apply only in the particular region in which the observations were made; there is no doubt that the relations of these species in other natural regions are different. For example, in the forested region to the north of the area in which this study was made, species of *Pisidium* occupy a much more prominent place in the pond fauna. Another example (Baker, 1928) is the characteristic molluscan association in the woodland

TABLE I

COEFFICIENTS OF ASSOCIATION

	L. stagnalis	*L. palustris*	*L. caperata*	*P. trivolvis*	*P. exacuous*	*P. umbil'us*	*P. arcticus*	*P. campestris*	*Aplexa hypnorum*	*Physa gyrina*
Lymnaea palustris	$\frac{49}{41.56}=1.18$									
L. caperata	$\frac{3}{20.08}=.15$	$\frac{62}{86.89}=.71$								
Planorbis trivolvis	$\frac{40}{12.92}=3.10$	$\frac{69}{55.91}=1.23$	$\frac{8}{27.02}=.30$							
P. exacuous	$\frac{16}{11.52}=1.39$	$\frac{64}{49.87}=1.28$	$\frac{12}{24.10}=.50$	$\frac{23}{15.50}=1.52$						
P. umbilicatellus	$\frac{0}{11.87}=0.0$	$\frac{29}{51.38}=.56$	$\frac{50}{24.83}=2.01$	$\frac{0}{15.97}=0.0$	$\frac{2}{14.25}=.14$					
P. arcticus	$\frac{11}{8.90}=1.24$	$\frac{49}{38.35}=1.27$	$\frac{9}{18.62}=.48$	$\frac{18}{11.98}=1.50$	$\frac{26}{10.69}=2.43$	$\frac{1}{11.01}=.09$				
Planorbula campestris	$\frac{5}{12.40}=.40$	$\frac{62}{53.64}=1.16$	$\frac{21}{25.92}=.81$	$\frac{12}{16.48}=.72$	$\frac{21}{14.88}=1.41$	$\frac{6}{15.33}=.39$	$\frac{17}{11.50}=1.48$			
Aplexa hypnorum	$\frac{1}{11.35}=.09$	$\frac{44}{49.11}=.90$	$\frac{35}{23.73}=1.47$	$\frac{4}{15.27}=.26$	$\frac{13}{13.62}=.95$	$\frac{23}{14.03}=1.64$	$\frac{8}{10.52}=.76$	$\frac{14}{14.65}=.96$		
Physa gyrina	$\frac{10}{4.71}=2.12$	$\frac{23}{20.40}=1.13$	$\frac{4}{9.86}=.41$	$\frac{6}{6.34}=.95$	$\frac{9}{5.66}=1.59$	$\frac{0}{5.83}=0.0$	$\frac{8}{4.37}=1.83$	$\frac{2}{6.09}=.33$	$\frac{2}{5.57}=.36$	

temporary ponds of Wisconsin, U. S. A., which is made up of the following species; *Lymnaea caperata, Physa gyrina hildrethiana, Aplexa hypnorum* and *Sphaerium occidentale.* Such diversity in the associations found in different natural regions tends to emphasize the need for further work, but does not detract from the significance of the results.

The areas of geographical distribution of the species treated in this account do not coincide, but the region here considered falls within the range of all of them. Moreover, in a broad sense the modes of life of all of them appear to be comparable.

The first point of interest in connection with the table given above is the wide divergence in the coefficients of association of the various species. Ponds are usually regarded as having a fauna made up of hardy eurythermal species of almost ubiquitous local and geographical distribution. In other words, that they are inhabited by organisms which have a wide range of tolerance of environmental conditions and which within broad limits can live in almost any body of fresh water. From the material presented here, it appears that this is not necessarily true, at least within the region under consideration, and that there is a high degree of specificity in the types of molluscan associations in different ponds. The coefficients of association of *Lymnaea stagnalis,* for example, which is commonly regarded as a typical pond snail, range from 3.10 with *Planorbis trivolvis,* to nil with *P. umbilicatellus.* Likewise, although both *P. trivolvis* and *P. umbilicatellus* are pond snails, they have never been found in the same pond.

There is little doubt that the occurrence and degree of desiccation in the ponds plays an important part in determining the presence or absence of certain molluscs. The climate of this area is of a semi-arid type, and there is usually a severe drought during the months of July and August. Many ponds are dry during this period, and it is noteworthy that *Lymnaea stagnalis* and *Planor-*

bis trivolvis have not been found in ponds in which this occurs. However, as far as other animals are concerned, these temporary ponds have a varied and abundant fauna, even though they contain water for only one or two months in each year (see Mozley, 1932). There are two species of molluscs, *Planorbis umbilicatellus* and *Planorbula campestris,* which occur only in habitats of this kind. The two species which are characteristic of permanent bodies of water (*L. stagnalis* and *P. trivolvis*) have a high coefficient of association (3.10), whereas the two species which are characteristic of temporary ponds (*Planorbis umbilicatellus* and *Planorbula campestris*) have a low coefficient (.39).

Thirty-eight of the 315 ponds examined in the course of this work were inhabited by a single species of mollusc. *Lymnaea palustris* was found as the sole molluscan inhabitant of nineteen of these ponds, *L. caperata* was found similarly in nine ponds, *Planorbis umbilicatellus* in four ponds, *Aplexa hypnorum* in four ponds, and *Planorbula campestris* in two ponds. There are few barriers to distribution in this region, and it seems reasonable to suppose that these occurrences of species singly represent the final stages of a process of elimination, so that the single surviving species of molluscs are the most hardy and well-adapted forms as far as the conditions in each individual pond are concerned. This being true, it appears that the two species which are found only in temporary ponds are not the most hardy forms in all such bodies of water, but only in some of them. This lends support to the view that even those ponds which are periodically dry may be grouped under several types, each with definite and diverse characteristics. This impression is further strengthened when the table of coefficients of association are examined in greater detail. It will be seen that there is a tendency for certain small groups of species to have mutually high coefficients, and there can be little doubt that this represents a close

affinity or parallelism in the life requirements of the species concerned.

The object of this note has been to point out that the association of these species of fresh-water mollusca with one another is not a haphazard affair, but is the result of definite, although complex reactions, and is capable of measurement and mathematical expression. It is also suggested as a working hypothesis that the position of these animals in their habitat may be much more precise and sharply defined than has previously been supposed; and that each one of them may occupy a particular little niche in the environment. *In different geographical areas this niche may be occupied by different species.* Whether this hypothesis will hold remains to be found out, but it is hoped that the method and point of view utilized in this account may prove to be of use in later work.

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A DEVELOPMENTAL ANALYSIS OF INHERITED SHAPE DIFFERENCES IN CUCURBIT FRUITS

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AMONG the most important as well as the most difficult problems of modern genetics are those concerned with the mechanism by which genes control development and thus produce the visible characteristics of the adult organism. In a field which is so beset with complexities an obvious procedure is to break the problem up into as many distinct components as possible, after which each can be separately attacked. The first step in such an analysis should evidently be to discover, by a purely descriptive study, what various developmental processes there may be which are concerned with the production of a given character and which are visibly different in material which is genetically different. When such are found, a genetic and physiological study of each will evidently be more hopeful of success than will an attack upon the whole problem at once. In the case of size differences in Cucurbit fruits a beginning at such a developmental analysis has been made. Here the extent of cell division, of cell expansion, of secondary wall formation and of growth before anthesis, all of which are important factors in determining mature fruit size, are found to be markedly different in different races and in most cases are inherited independently of one another (Sinnott, 1936).

It is with shape characters, however, which are among the most difficult traits for genetic investigation, that such a method of developmental analysis proves especially valuable in breaking down the major problem into a series of separate ones. Shapes are particularly hard to deal with since they elude precise measurement and are usually described only by a series of terms, necessarily inexact. Relatively simple shapes, such as those of most fruits, can

be reduced in part to a measurable basis by determining for them an index or ratio between the two major dimensions—length, taken along the main axis, and width, the maximum dimension at right angles to this. To express all the details of an organic pattern in quantitative terms, however, is almost impossible. On the other hand, traits of shape have an advantage over many others for developmental study in that almost their entire history can usually be followed. This is especially easy with fruits, where the changes in size and shape from the earliest primordium, microscopic in size, to maturity, may be measured.

The members of the Cucurbitaceae or gourd family offer especially useful material for such work, since they display a very great variety of size and shape in their fruits and since a beginning has already been made among them on a study of fruit shape inheritance. The value of the method of developmental analysis as an aid in such genetic investigations is shown by the fact that in this family four quite distinct types of shape determination, each genetically controlled, have already been found. These will be described briefly in the present paper.

In the simplest of these types the difference in shape index is present at the very beginning of development and is visible as soon as the ovary primordium is differentiated in the small mass of cells from which a pistillate flower will arise. Such differences are clearly controlled by genes, for if a race of *Cucurbita Pepo* in which fruit length and width are essentially equal (a "sphere" type) is crossed with one having a much flattened "disk" fruit, the F_2 shows a clear segregation into $\frac{3}{4}$ disks and $\frac{1}{4}$ spheres. Evidently a single major gene difference is here involved. In the races of *C. Pepo* studied the shape index becomes slightly flatter as growth proceeds, but there is no profound change during the course of development. That genes here control a definite relationship and not one or more dimensions alone is indicated by a number of lines of evidence (Sinnott, 1935*a*). Such a shape difference

expresses itself uniformly in fruits differing widely in size, and its genic basis seems quite independent of that which determines the actual bulk of the organ. The essential feature in this type of shape determination is that the genes which control it produce their chief visible effect only in the very earliest stages of development.

In the second type of shape control, differences in index of the mature fruit are not established in the earliest primordia but result from a constant difference in growth rate between length and width during the entire course of visible development (Fig. 1). If length is plotted against

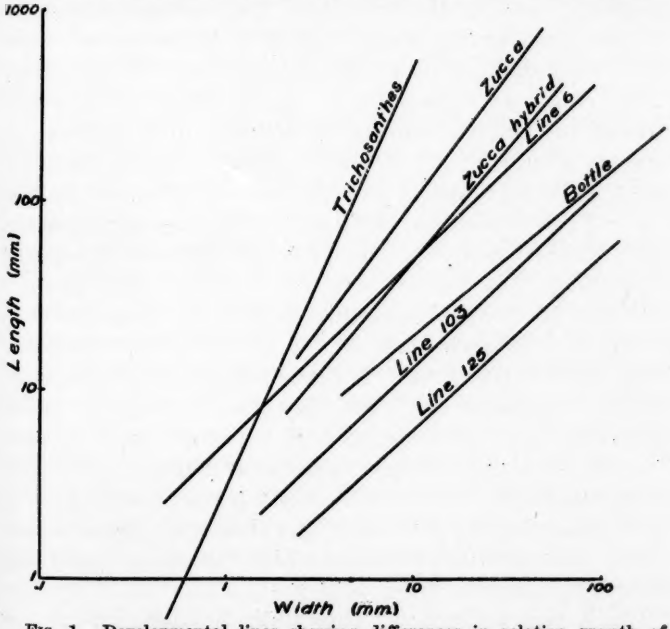

FIG. 1. Developmental lines showing differences in relative growth of length and width in various cucurbit fruits. Lines 6, 103 and 125 are *Cucurbita Pepo* ($k = .95$) and differ essentially only in level (value of b). "Zucca" ($k = 1.3$) and "bottle" ($k = .8$) are varieties of *Lagenaria vulgaris*, and the hybrid ($k = 1.2$) is between zucca and a type similar to bottle. *Trichosanthes* ($k = 2.2$) is the "snake gourd."

width, both logarithmically, in a series of stages from ovary primordium to mature fruit, a straight line results.

Logarithmic plotting makes it possible to compare the true or geometric rate of increase of the two dimensions, and the slope of the line measures the relation between the two rates.[1] In the races of *C. Pepo* studied, the slope of this line (the value of the relative growth constant k) is about .95, showing that there is no very radical change in index during development, but that length does not increase quite as fast as width. Large fruits are therefore slightly flatter than small ones. In races of *Lagenaria vulgaris,* however, which includes many of the cultivated gourds, there is greater variation in this respect. Among the so-called "bottle" gourds, width grows considerably faster than length, the slope of the line being about .8 in the varieties studied. In the "Hercules club" or "zucca" type, on the other hand, length grows faster than width, the relative growth constant having a value of 1.3, so that the fruits become considerably more elongate as size increases rather than flatter, as do *C. Pepo* and the "bottle" gourds. A cross between the "zucca" and the "sugar trough" gourd (the latter resembling the "bottle" in its development) shows a relative growth constant intermediate in value between those of the parents but nearer to that of the "zucca." The most extreme case is that of the truly remarkable Indian "snake gourd" *Trichosanthes,* which may reach a length of over two meters with a diameter of only four centimeters. Its relative growth constant for length-width is about 2.2. The tiny ovary primordia, when they first appear, are not much longer than wide, but so much faster is length growth than width that at maturity the fruit may have an index of 50!

Each type of cucurbit apparently has its own specific relative-growth constant for length and width and these

[1] The advantages of this method of measuring growth relationships have been fully discussed by Huxley (1932). The degree of relationship may be indicated by a constant, k. If x in this case is width, y length and b a constant denoting the value of y when x is unity, then $y = bx^k$, or $\log y = \log b + k \log x$. If the change in relative growth of x and y is a constant one, a straight line will result when they are plotted against each other logarithmically, and the slope of this line will measure the value of k.

are undoubtedly genically controlled, although direct evidence of this is as yet lacking.[2] Kaiser (1935) has recently shown that fruit shape differences in *Capsicum* also result from differences in relative dimensional growth, but in this genus all types are approximately alike until flowering, after which divergences appear.

In this second type of shape determination, gene control is evidently exercised upon relative growth throughout the course of development in contrast to the first type described, in which it was exercised only during the previsible stages of growth while the ovary is differentiating. In the first type, genes affect the *level* of the length-width line; in the second they affect its *slope*. In terms of the formula, they modify the value of b in the first type and of k in the second.

A third type of shape index determination is produced by any genetic change which affects size, if the growth rates of length and width are unequal. This is well illustrated by comparing two races of bottle gourds, the "miniature" and the "giant." The former at maturity has a length of about 10 cm and a width of about 7, while an average fruit of the latter is 23 cm long and 20 cm wide, so that the smaller one has an index of 1.4 and the larger one of 1.15. If we measure developing ovaries and young fruits in both types, however, and plot length against width as before, all the points fall along the same straight line, the slope of which has a value of .8 (Fig. 2). The "miniature" type, which reaches maturity at a rather small size, resembles a young, partly grown fruit of the "giant." Since width is growing faster than length, however, the fruits grow markedly flatter with increasing size, and the mature "giant" thus has a very different index from the mature "miniature." If one could develop a form like these except that it grew still larger, it would be still flatter; and if maturity occurred at an even smaller size than in "miniature" the fruit would be still more elongate. Fig. 3 shows the differences in shape

[2] This should be provided by the F_2 from the zucca × sugar-trough cross here described.

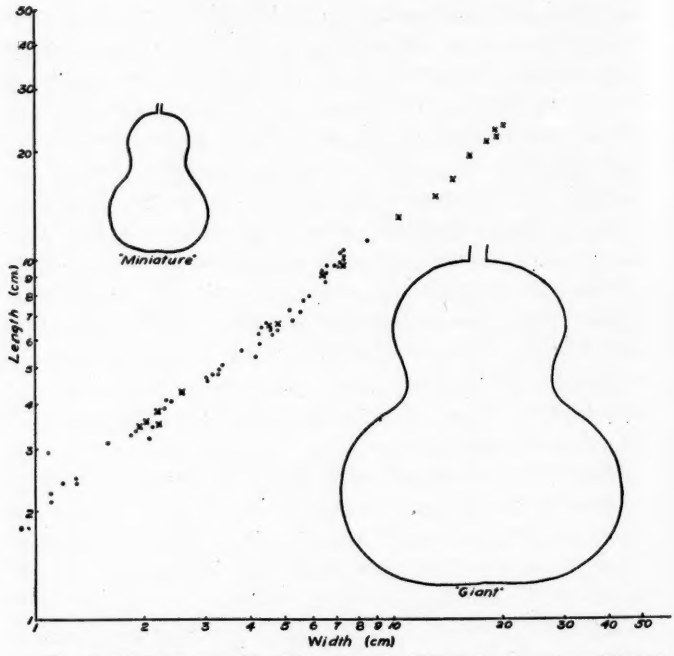

FIG. 2. Relative growth of length and width in large and small bottle gourds (*Lagenaria*) from early primordia to maturity. "Giant" represented by crosses, "miniature" by dots. Inserted profiles of mature fruits show relative size and shape.

which would result if growth were to stop at each of four points along this developmental line.

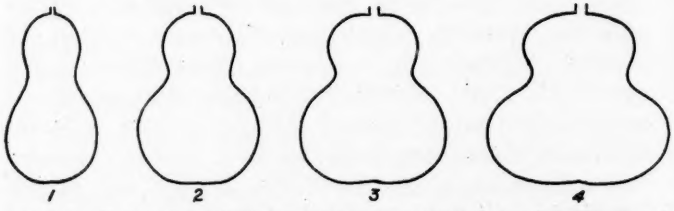

FIG. 3. Fruits taken from four points along the line shown in Fig. 2, and reduced to the same length so that shape changes induced by size differences may be directly compared. 1, partly grown "miniature"; 2, mature "miniature"; 3, mature "giant"; 4, hypothetical "giant" fruit which had grown to twice its normal length.

It is evident that such differences in index are quite dissimilar in character to the others described. These bottle gourd types have the same slope and the same level for their length-width developmental line and differ only in their size at maturity, that is, the point along this line where growth stops. Genes which produce differences in size will therefore necessarily bring about differences in shape index wherever growth of the two dimensions is not equal. These two races of gourds may thus be regarded as differing primarily in size and only secondarily in shape. The important rôle of size changes in altering form, where the relative-growth constant is not unity, has recently been shown for evolutionary series in animals by Robb (1932) and Hersh (1934) and for plants by Kaiser (1935).

Shape index of the cucurbit fruit at maturity may thus be affected in three distinct ways, through differences in (1) initial index of the ovary primordium, (2) relative rates of growth in length and width during development, and (3) fruit size at maturity, in cases where dimensional growth rates are unequal. Differences in each of these types may occur independently of the others, and they may be combined in all possible ways.

The shape index, however, is by no means a complete expression of shape differences. Fruits with the same index may differ markedly in the outline or profile which they present and which may perhaps best be described as their *pattern*. Thus in one line where the fruit is three times as long as wide the maximum width may be approximately half way from the tip to the base, with a gradual taper in both directions, whereas in another fruit of exactly the same index the maximum width is found in a relatively short, swollen zone toward the apex of the fruit, and the basal region is constricted to a long, tapering "neck" (Fig. 4, *1* and *2*). In *Cucurbita Pepo* (and in some other species) there is a great variety of patterns. Although a quantitative description of each may be given,

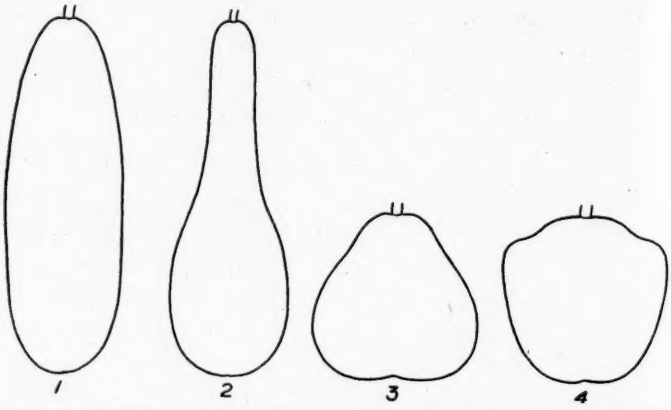

FIG. 4. Pattern differences in fruits with the same index. 1 and 2, two types of *Cucurbita Pepo*, both with an index of 3. 3, line 103; 4, line 22, both with an index of 1.

it is necessarily complex, and the pattern can best be represented by an actual profile or picture.

A genetic analysis of such patterns is not simple, but there can be no doubt that they are inherited, since specific ones characterize specific inbred lines of *C. Pepo* and since there is an evident segregation, although usually a complex one, in the F_2. The simplest case yet analyzed concerns two patterns which express themselves in essentially isodiametric form in lines 103 and 22 (Fig. 4, *3* and *4*). In the former the fruit is broadly pear-shaped, with the widest diameter nearer the tip than the base (stalk end). The latter is "high-shouldered," with the widest diameter much closer to the stalk end than to the tip. When the two lines are crossed the F_1 is a flat disk and the F_2 gives approximately 9/16 disks, 6/16 isodiametric fruits, and 1/16 elongates, due to the segregation of two factors determining index level. Among the isodiametric types in F_2, however, the two parental patterns may be distinguished, with the pear-shaped type in a minority (about one fourth) of the individuals. This suggests that a single major gene is concerned with the distinction between these two patterns. The two are indistinguish-

able in the disk fruits and hard to separate in the elongates. Pattern is apparently determined independently of index, for fruits with the same index may have very different patterns, and what is clearly the same fundamental pattern may be present in fruits of very different indices. There is evidence that change in index resulting from genetic segregation modifies the expression of pattern in a regular and predictable fashion (Sinnott, 1935*b*).

Practically nothing is known at present as to the developmental mechanisms concerned in any of these four types of shape determination. That they involve primarily a control of cell number rather than cell shape is indicated by the fact that in all fruit shapes the cells of comparable tissues are essentially the same in shape. Differences in organ shape are thus due to the relative numbers of cells along certain axes as compared to others, and thus presumably result from genetic control of the planes of cell division. Differences in index, resulting from the way in which the material of the fruit is pulled out or compressed, so to speak, along its axis, evidently involve the general problem of polar growth and may perhaps be related to the concentration and polar distribution of growth hormones. Pattern, on the other hand, involves a more complex series of growth correlations by which specific changes in diameter along the axis are determined. We may ascribe this control to an axial gradient or a morphogenetic field, but these concepts are as yet too vague to be very helpful.

SUMMARY

Fruit shape in the Cucurbitaceae is inherited, and there are at least four quite distinct types of shape determination, as follows:

(1) Differences in shape index (ratio of length to width) which are established in the earliest visible primordia and may persist unchanged throughout development.

(2) Constant differences in growth rate between length and width which occur during the entire course of development, causing a progressive change in index. The relative growth of these two dimensions may be measured by a constant. In the types studied this varies from .8 (length growing .8 times as fast as width) to 2.2 (length growing 2.2 times as fast as width).

(3) Differences in shape index at maturity which necessarily result from differences in size in all cases where the relative-growth constant is other than 1. Where shape index is progressively changing, the point at which growth of fruit stops (its mature size) will affect its index.

(4) Differences in pattern or profile, which are more complex than those involving index alone. Specific patterns are inherited and are determined independently of differences in index.

All four of these aspects of shape are independent of each other in inheritance. The genes which control them evidently differ in the time at which the major effect is produced and in the character of the effect itself.

A purely descriptive developmental analysis is a necessary preliminary to any complete genetic or morphogenetic study of characters of size or shape.

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SHORTER ARTICLES AND DISCUSSION

A NEW METHOD OF SYNTHESIZING PURE-BREEDING TYPES WITH EXTRA CHROMOSOMAL MATERIAL IN DATURA[1]

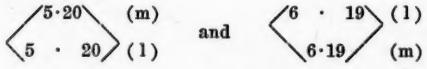
In previous methods of synthesizing pure-breeding types in Datura,[2] fragments of chromosomes have been added to the ends of one or more of the necessary chromosomes of the plant and this extra chromosomal material has brought about the peculiarities in appearance of the new races which were thus produced. In these cases the translocated fragments could be identified by matching them up with known ends of tester chromosomes. The present method involves adding the extra material inside the chromosome and not at the ends. This has been accomplished in the following fashion. It will be remembered that the chromosomes are designated by numbering their ends and that they can be matched up like dominoes when examined under the microscope at certain stages of development. Our standard Line 1 has among its chromosomes a large "l" chromosome 5·6 and a small median "m" chromosome 19·20. By the action of x-rays, segments of these two chromosomes were interchanged so that a prime type[3] race (PT 39) was formed which had an m-sized 5·20 and an l-sized 6·19 chromosome. Through the action of radium treatment, another prime type (PT 53) was obtained which had these same two chromosomes interchanged, with this difference, that the breaks in the 5·6 and 19·20 chromosomes occurred at different loci. As a result the 5·20 chromosome was now l-sized and the 6·19 chromosome was m-sized. In the second case, the ends of the modified chromosomes are the same as in the first case, but the sizes are reversed. In PT 39, the m-sized 5 · 20 plus the l-sized 6 · 19 chromosomes have the same genic content as the l-sized 5 · 20 plus the m-sized 6 · 19 in PT 53.

[1] Paper presented at meeting of the American Philosophical Society, April 23, 1936.

[2] A. F. Blakeslee, *Proc. of the Sixth International Congress of Genetics*, 1: 104–120, 1932; A. F. Blakeslee, A. Dorothy Bergner and Amos G. Avery, *Proc. Nat. Acad. Sci.*, 19: 115–122, 1933; A. F. Blakeslee, *Jour. of Heredity*, 25: 80–108, 1934.

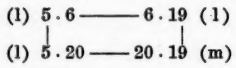
[3] A. Dorothy Bergner, S. Satina and A. F. Blakeslee, *Proc. Nat. Acad. Sci.*, 19: 103–115, 1933.

By crossing together PT 39 and PT 53, an F_1 was obtained which showed two unequal pairs:

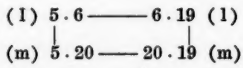


The upper two chromosomes are those of PT 39, the lower two are those of PT 53. Starting with this F_1, it was possible by proper breeding procedure to eliminate the m-sized 5·20 and 6·19 chromosomes and to replace them by the larger l-sized 5·20 chromosome from PT 53 and the l-sized 6·19 chromosome from PT 39. These plants therefore had two l-sized 5·20 and two l-sized 6 · 19 chromosomes but no 5 · 20 nor 6 · 19 chromosomes of m-size. The total genic content of these four l-sized chromosomes is more than that of either prime type or than the F_1 between them. Therefore this new type shows peculiarities in appearance to be expected from the extra chromosomal material which is present inside the modified chromosomes. Such plants show the usual 12 homologous pairs of chromosomes, but in place of the 8 chromosomes of relatively large size and the 4 of relatively small size characteristic of normal plants, they have nine large and only three small chromosomes.

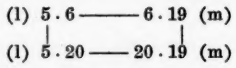
Further evidence supporting our interpretation has been obtained from a back-cross of the new type to our standard Line 1, which shows at the first reduction division a circle of 4 chromosomes arranged as follows:



In this circle there are three large to only one m-sized chromosome. When PT 39 is crossed to Line 1 a circle of 4 is formed as follows:



When PT 53 is crossed to Line 1 the circle is as follows:



Both of these latter cases differ from the back-cross to the new type in having only two l-sized chromosomes.

The new pure-breeding type has been synthesized without increasing the number of chromosomes, or the number of ends

which show attachments in reduction divisions. The excess material within the chromosomes brings about effects upon the appearance of the plant due to chromosomal unbalance. Such synthesized races resemble species formed in nature in that their chromosomes appear normal and their deviations from the types from which they arose are brought about by additions of blocks of genes rather than by changes in single genes. For this reason many characters of the plant are altered rather than only a single character.

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THE "RESTING" NUCLEUS

For many years the nucleus dividing by mitosis has been known as the kinetic or the karyokinetic nucleus, while the interkinetic or non-dividing phase has been termed the resting nucleus. This latter term is relatively accurate in a descriptive morphological sense, but the implication of non-activity is correct only in the sense that the constituents of the nucleus are not undergoing movement in space relative to each other, with the possible exception that karyomeres, as in Ascaris, and nucleoli in general may fuse or fragment during interkinesis. The chromosomes, however, reappear at the prophase of one division in the same positions that they occupied at the telophase of the preceding one. On the other hand, there is no doubt that the non-dividing nucleus is an active body and the "resting nucleus" as a general term is a misnomer. As long ago as 1855 Rabl emphasized that cytologists should free themselves from the "historischen Vorstellung" that the "resting nucleus" is in a state of rest.

Sharp (1935) has recognized this and suggests that the resting nucleus be known as the metabolic nucleus. This suggestion, while an improvement, is not completely satisfactory, for "metabolic nucleus" implies that the nucleus is primarily active in connection with the nutrition of the cell. This is too limited a conception, and it is felt that a term is needed that will imply that the "resting" nucleus is internally immobile, in contrast to the kinetic or dividing nucleus, and yet that it is active in a much wider sense than is implied in the word "metabolic."

How fundamental an activity there is associated with the constituted non-dividing nucleus is shown by the complete dependence of cell structure and function upon the persistence of the constituted nucleus, and the degeneration or cessation of such structure and function during the kinetic phase. This correlation is shown most strikingly in fission in the hypotrichous ciliates. Wallengren (1901) for Stylonichia and Holosticha and C. V. Taylor (1928) for Uronychia showed that binary fission is accompanied by a resorption of all motor organellae and the outgrowth of a new set in each daughter cell. At a critical stage after karyokinesis, but before cytokinesis is completed, three sets are present, one old and degenerating, two new and developing. Comparable phenomena are typical of certain tissues of Metazoa: Tissue cells of the Metazoa fall into three general categories: (1) unspecialized multipotent cells able to divide freely by mitosis, (2) highly specialized cells able apparently to divide only by amitosis [(red blood corpuscles of Amphibia) Charipper and Dawson (1928), Ferrari (1931) (bone cells) Bast (1921)], or not at all (retinal cells, etc.), and (3) cells of an intermediate degree of differentiation that behave during mitosis in a manner strictly comparable with the ciliate Protozoa. The ciliated epithelial cells of the frog esophagus (Kindred, 1927) divide by mitosis but with a temporary loss of ciliated structure. When ciliation was retained, division, according to Helvestine (1921), was amitotic in the ciliated cells of the gill filaments of Cyclas. It may be added parenthetically that many cases described as amitosis may in reality be aberrant or atypical mitosis; amitosis appears to be confined either to degenerating or to old, fully differentiated cells. Intestinal epithelial cells of the lamprey, trout and frog (Cohen and Berrill, 1936) lose their characteristic columnar form with the onset of mitosis and become spherical, regaining their typical shape after the nuclear membrane has been re-formed. In the case of the lamprey, the cilia borne by these cells are lost during mitosis and are re-formed in the daughter cells.

Peter (1924) from studies on secretion and resorption in Salamander larvae has confirmed the earlier conclusions of Meves (1899) on secretory cells and has stressed the negative correlation between mitosis and physiological activity: "eine Zelle, die sich indirekt teilt, arbeitet nicht," he concludes. Jolly (1904) noted that the haemoglobin content of red blood corpuscles of Triton disappears with the onset of mitosis, and Manwaring in 1904

(cited by Ortiz-Picón, 1935) established the absence of glycogen and fat in liver cells dividing mitotically, though neighboring non-dividing cells may be full of these substances. Ortiz-Picón, reviewing these observations, says that they probably indicate that cell-products are used up during mitosis and that the dividing cell is unable either to manufacture more or to draw them from the surrounding tissue; they indicate that "die Zelle verliert also während des mitotischen Vorganges ihr Arbeitsmaterial, das der Ausdruck ihrer eigenen specifischen Funktion darstellt." Many further similar data are given by Wassermann (1929, pp. 495–507). In his own studies on the stellar cells of the mouse liver Ortiz-Picón confirmed Peter's conclusions for inter-cellular processes, but found that the intra-cellular activities of vacuolar digestion and excretion may go on during mitosis; if these are considered as passive processes Peter's generalization, he concludes, is valid.

It is also significant that during development, whether sexual or asexual, precocious structural differentiation of cells coincides with a cessation of division, while in tissues where division proceeds until minimal cell sizes are attained cyto-differentiation is postponed until cell divisions are ending (Berrill, 1935). Again, secretory granules in intestinal epithelial cells of the frog tend to disappear entirely during mitosis (Cohen and Berrill, 1936), suggesting a cessation of secretory activity. Thus there is considerable evidence that cell structures and form can only be developed during the inter-kinetic phase of the nucleus, and that during mitosis such features are lost and are re-formed only when the daughter nuclei are reconstituted.

There is further experimental evidence to show that specialized cell functions are likewise suspended during mitosis. Proliferating cartilage cells in tissue culture lose their distinctive character and fail to form the intercellular matrix (Fischer, 1922). When at the center of transplants mitosis tends to cease, the cartilage cells re-form the matrix (Strangeways, 1924). Fischer and Parker (1929) have shown that fibroblast types of cells, even though grown *in vitro* for 7 to 9 months, do not lose their capacity for tissue organization or for the assumption of their special function. It is the process of active cell-proliferation that inhibits these capacities. When, through the use of pure blood plasm without the introduction of other substances, cell-division is sup-

pressed they form tissues similar to those they would have formed in the body.

It was suggested recently (Berrill, 1935, p. 358) that "the activity responsible for the maintenance of certain kinds of cytoplasmic structure, associated with the so-called resting nucleus, be known as morphenergesis." In extension of this it is now suggested that the term "resting nucleus" should be definitely discarded and that "energic nucleus" (*Energeia* f. En(*erges*) f. *ergon* work) should be used in its place. The energic nucleus is a stationary working unit, in contrast to the kinetic nucleus in which the constituent chromosomes are in a state of movement. Sachs (1892) used the term "Energide" to designate the physiological unit, as he conceived it, of the nucleus and the surrounding cytoplasm which it influences; the etymology and underlying idea is the same as in our suggested name for the "resting" nucleus. The term "energic nucleus" implies the existence of an activity that includes the development and maintenance of all cytoplasmic structure and function, and affords a suitable contrast to the other type of nuclear activity associated with mitosis and well expressed by the established term "kinetic nucleus."

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FORM AND SIZE VARIATION IN A SPATHIDIUM BELIEVED TO BE *SPATHIDIUM SPATHULA*

INDICATION of the rarity of *Spathidium spathula* is seen in the scarcity of recorded observation in the protozoan literature of this country since the papers of Woodruff and coworkers in the early part of the last decade. Because of the infrequency of occurrence, rediscovery of the organism and a structural comparison with forms previously described in distant localities deserve report.

Three specimens of this predatory ciliate were recovered during a series of routine examinations of numerous culture samples taken out-of-doors in spring. Each animal was characterized by flask-like shape with narrowing neck, obliquely truncate anterior end, thickened lips and broader, rounded posterior region. Cilia were fine, somewhat coarser around the anterior border and evenly distributed. The body was delicately but visibly striated.

Within the cell the posteriorly placed contractile vacuole was clearly evident, measuring 26.64 μ in length in one instance. The cord-like, typically twisted macronucleus showed plainly upon application of the vital dye safranin in 1–1,000 concentra-

tion. Very few food vacuoles were present, and the protoplasm was finely granular.

Organisms A and B, quieted by the stain, retained body shape very perfectly at the given strength used. They measured 99.96 μ and 156.5 μ in length, respectively. C, notable for its great extension, resembled to a marked degree Fig. 131 of Conn (1904–1905) in relative proportions and general outline. The width of the body was very nearly the same throughout the cell, narrowing in the "neck" region slightly to give the usual graceful symmetry. After fixation in a very much diluted mixture of 0.1 per cent. magnesium sulfate and concentrated mercuric chloride, the shape and size were retained so far as could be detected. The whole animal was 228.48 μ in length and was 33.3 μ at its widest point, about two thirds of the way back.

A comparison of sizes in these three Spathidia with figures secured by other workers demonstrates the range of variation existent in the group. Bütschli gave up to 240 μ for length. Dujardin, according to Moody (1912), found 180–240 μ as an average, whereas Maupas concluded 160 μ was the maximum. Moody obtained a mean length and width of 110.5 μ and 35.2 μ, respectively, the smallest of her forms being 73.5 μ long and 21 μ wide, the largest reaching 157.5 μ in length and 57.5 μ in diameter.

Moody's smallest specimen was considerably shorter than any of the three observed herewith, her longest practically that of B but longer than A and considerably shorter than C. Dujardin, however, seems to have recorded a much greater length than C, indicating that the elastic properties of the protoplasm were enormous, one case having been seen to stretch to five times its ordinary length. Moody observed a single instance of doubling it. Woodruff and Spencer (1922) agree that there was considerable power for extensibility, but did not note such extremes.

Inasmuch as all three individuals here were free to move, unhampered by debris or obstruction, and since it was not noticed that there was any variation in length during the period of observance it is likely that these were fairly constant, though not necessarily average nor typical, group-size proportions. It should be mentioned that there may have been a very rapid response of the much attenuated third member to the chemical stimulation of the fixative, resulting in a possible slight increase in extension.

Variation in the lengths of A, B and C is quite obvious. This merely emphasizes what other American workers have clearly

demonstrated in their pedigree lines. Physiological factors within and without the cell modified its size greatly within the species. The inconstancy is not unusual, therefore, nor does it imply systematic differences. Starved specimens became elongated in the isolated strains, the protoplasm changing in consistency and granularity. The cytoplasm here was not filled with inclusions nor coarse, deeply staining food vacuoles and metaplasmic bodies, as is so often the case with well-nourished ciliates. It was, instead, finely granulated and fairly clear. This is entirely in keeping with the scarcity of available materials for nutrition in the water sample taken.

In such instances of form and size discrepancy pedigree cultures should furnish the safest means for determining essential inherited differences. The approach to the problem of species differentiation and proper taxonomic position of the major divisions and minor subdivisions of the protozoa will be made best perhaps through the laborious routine of rearing isolated lines sufficiently long to determine what characters are stable and what are due to merely transitory fluctuations brought on by environmental factors. Even then the task will not be simple to carry through, for, as is well known, a basic feature such as the nucleus may vary radically within the genus and, to some extent, the species. Stentor, for example, has a sharply defined "chain of beads" in one group and a positively rounded nucleus of the vegetative type in the other. Recent work undertaken on some of these characters indicates that the shape of cellular inclusions, transitory and permanent, for a given wild population, is far from perfectly constant. Until a great many more facts have been accumulated on the genetic constitution of the protozoan cell and the inheritance over many generations of characteristics which may safely be considered reasonably stable, a species classification of widely varying random examples coming from a wild population is bound to be marked by uncertainty.

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ZOOGEOGRAPHICA—A REVIEW

ALTHOUGH *Zoogeographica* first appeared in January, 1932, and completed its second volume in March, 1935, we have good evidence that it has to date escaped the attention of certain American students in closely related fields. This journal announces itself as an international review for comparative and causal animal geography; it is edited by Fridthjof Ökland of Oslo with the aid of Svan Ekman of Upsala and Richard Hesse of Berlin. It is published from the press of Gustav Fischer of Jena; the two volumes that have appeared have contained slightly over 600 pages each. Approximately a half volume was announced for 1935 at a total cost of not more than 25 RM ($10 at January, 1936, exchange!), with the recently arranged discount of 25 per cent. Publication of papers may be in German, English or French, but to date the great majority of articles have been in German and no American or English students of animal distribution have contributed to the journal.

An outstanding service is the ambitious attempt by Bernhard Rensch of Berlin to bring together annually and to characterize briefly the publications in animal geography for a given year. In Volume I he cites (pp. 413–448) 151 titles that appeared in 1931 and in Volume II he continues with 225 titles (pp. 410–453) mainly from 1932 but with a few previously undiscovered titles from 1931. From the nature of the case the lists are incomplete; purely taxonomic or ecological studies with slight zoogeographic value are purposely excluded. The titles are grouped according to zoogeographic regions.

It is difficult to summarize or to characterize the contents of the thirty-five more or less diverse articles from twenty-five different authors which have appeared in this journal. Some of the tendencies can be seen from the following somewhat hasty and partially subjective analysis of major features of the different articles. The following topics are given more than casual attention:

Caves	1	Marine	7
Glaciation	6	Fresh water	8
Local zoogeography	9	Lake Baikal	1
Faunal affinities	13	Land animals	17
Migration	2	Europe	17
Evolution	2	Asia Minor	3
Human influence	1	Siberia	2
Land bridges	1	Africa	2
Taxonomic emphasis	17	S. America	1

Ecological emphasis	12	N. America	1
Dealing with restricted taxonomic group or even with a single species	9	Southern continents	1
		Holarctica	1
Climatic factors	4	Parasites	1
		Bibliography	2

In the thirty-three papers, after exclusion of the two bibliographic summaries, two deal with animal life in general, seven with vertebrates and twenty-four with invertebrates. The detail with which the distributions resulting from the Pleistocene ice age have been examined in Europe is notable, and five papers are devoted to this topic, among which that of Holdhaus on the cave beetles and one by Heberday on the carnivorous beetles of the Alps, with their carefully mapped evidence, require especial mention.

Marine zoogeography is represented by two important papers by Dr. Ekman which discuss the age of the Atlantic fauna, a problem which enters into the more general one of transoceanic continental connections or of continental drift; by two papers by Berg on discontinuous marine distributions, one discussing "bipolarity," the other the less known separation of the North Atlantic and North Pacific faunas by the Arctic fauna proper; and by papers on copepods, on the general problem of regional subdivision of the oceans and on littoral distribution as affected by ecological factors.

Among other more notable papers, that of Frieling on the spread of the diver, *Podiceps nigricollis nigricollis*, in Central Europe in recent years appears to be especially well documented with diagrams illustrating directions and rates of dispersal and ecological differences in habits; the modern spreading of this species appears to be taking place by discontinuous irruptions.

Mertens's account of the distribution of the sea-snakes is an example of a summary of the distributional relations exhibited by a compact group, in this case a complete family. Among regional studies, the list of the Lepidoptera of Palestine is an ambitious one, analyzing the distribution of the 1,335 species with reference to ecologic factors as well as to faunal relations.

Fresh-water animals are dealt with in eight papers. Of these, Dr. Berg's review of the distribution of the fresh-water fishes of Europe is an excellent summary, in which the fauna is analyzed into provinces and districts. It is illustrated with maps, many of which exhibit interesting discontinuous ranges. A second paper by the same author discusses the supposed marine origin of the

animal life of Lake Baikal, with the conclusion that it is an ancient fresh-water fauna, and marine in origin only as this is true of the fresh-water invertebrates as a whole.

The distribution of land vertebrates, which has been the dominant topic in North American zoogeography, is distinctly secondary in importance in these volumes, which tends at least to equalize these interests. There appears also to be a definite trend away from the uncritical acceptance of transoceanic connections between the continents.

While some of the studies would have fitted equally well into one of the current ecological journals, the majority of them would probably not have been welcomed there in their present form and emphasis; hence it appears that this new journal fills a niche in biological publications which has hitherto been unoccupied.

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A SINGLE THEORY FOR THE PHYSIOLOGY OF DEVELOPMENT AND GENETICS

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I. INTRODUCTION

PRESENT-DAY biological thinking tends to regard the physiology of development and genetics as two separate and even opposing fields. This is shown positively by those embryologists who insist that the phenomena in the progressive differentiation of the embryo from the egg are irreconcilable with those of Mendelian genetics, and negatively by the larger group of geneticists who, arguing for the control of the stages in differentiation during development by the genes, fail utterly to establish their claim. Thus, the embryologist, Lillie (1929, p. 528), speaks of

> a division of the phenomena of the life-history into three major components: morphogenesis, embryonic segregation and genetics. Morphogenesis is the development of form, organs and the special functions. Embryonic segregation concerns the origin of specific potencies as defined. Genetics concerns the determination of phenotypic characters throughout the life-history.
>
> The independence of morphogenesis and embryonic segregation is dealt with in the present essay. The independence of genetic phenomena from the other two is treated slightly in "The Gene and the Ontogenetic Process" (Lillie, 1927), but requires farther development. Embryonic segregation and genetics are the only two aspects of the life-history for which we have accurate criteria—for embryonic segregation that of self-differentiation, for genetics that of Mendelian segregation and assembling of genes. They are independent variables of the life-history.

So earlier Conklin (1924, p. 600 *ff*):

> The genes or Mendelian factors are undoubtedly located in the chromosomes, and they are sometimes regarded as the only differential factors of

* The cost of this supplement is in part defrayed by the author.

development, but if this were true these genes would of necessity have to undergo differential division and distribution to the cleavage cells, as Weismann maintained. Since this is not true it must be that some of the differential factors of development lie outside of the nucleus, and if they are inherited, as most of these early differentiations are, they must lie in the cytoplasm.

Among geneticists, Goldschmidt (1932) and Morgan (1926) have urged with complete lack of conviction the union of the physiology of development with genetics. Even less successful have been the attempts of others. Jennings (1930), for example, has recourse only to dogma wholly at variance with the facts of embryology and Demerec (1933, p. 370 *ff*) seeks refuge in dicta as the following:

This self-propagating property of the gene is one of its important characteristics.

. . . each one [gene] individually and all of them together having an almost magic power of governing life processes of cells in which they are located, and therefore of governing life processes of the organism of which these cells are an integral part.

The attempts of geneticists to bring the physiology of development and genetics into one category are predicated upon failure because of their conception that every cell in the body of the organism has the entire complex of genes. Says Morgan (1924, p. 717):

The embryological evidence points unmistakably to the conclusion that at each cleavage of the egg the chromatin material is divided equally, and that every cell of the body receives the sum total of the genetic materials carried by the chromosomes. This evidence is in full accord with the conclusions drawn above, for the whole genetic complex is present in every part of the body at all times. Whether all the genes are functioning all the time, or only begin to function when in the course of the progress of embryonic development new structures arise, we do not know, but however this may be, it is evident that since all the genes are present, the development of every part may be affected by the presence of all or of any of them.

In another place Morgan (1926, p. 491) says:

Except for the rare cases of plastid inheritance, the inheritance of all known characters can be sufficiently accounted for by the presence of genes in the chromosomes. In a word, the cytoplasm may be ignored genetically.

It is this conception which embryologists like Conklin and Lillie insist renders genetics valueless for an expla-

nation of the physiology of development. Says Conklin (1924, p. 600):

> The evidence is conclusive (1) that genes, or Mendelian factors, are located in the chromosomes; (2) that in mitosis each chromosome, and presumably each gene, divides equally and the halves are distributed equally to the daughter-cells; (3) that consequently all cells of an organism have typically the same chromosomal constitution, unless the chromosomes become secondarily modified by different kinds of cytoplasm or different environmental influences. How then is it possible to correlate chromosomal inheritance and cytoplasmic differentiation? How can identical genes in every cell lead to the multitudes of inherited differences in differentiated cells?

And Lillie (1927, pp. 365 and 367):

> It is apparently not only sound, but apparently almost universally accepted genetic doctrine to-day that each cell receives the entire complex of genes. It would, therefore, appear to be self-contradictory to attempt to explain embryonic segregation by behavior of the genes which are *ex. hyp.* the same in every cell.
>
> . . . The progress of genetics and of physiology of development can only result in a sharper definition of the two fields, and any expectation of their reunion (in a Weismannian sense) is in my opinion doomed to disappointment. Those who desire to make genetics the basis of physiology of development will have to explain how an unchanging complex can direct the course of an ordered developmental stream.

Embryologists on their side no less rigidly adhere to their doctrine of the cytoplasm as the component of preeminence in development. This is shown by Conklin and by Lillie in their respective conceptions of the rôle of embryonic segregation in development. And yet, both geneticists and embryologists have moments when they lapse. Thus, geneticists can not entirely overlook the cytoplasm as the source of origin of the embryo. Says Morgan (1924, p. 728):

> These statements are not intended to imply that the cytoplasm is a negligible element in the development of the organism. On the contrary, the developmental processes appear to be entirely dependent on it. How the genes in the chromosomes produce the effects in and through the cytoplasm we do not know. There is no evidence whatever to show that materials produced by the genes pass out and make the cytoplasm. For all we know to the contrary, most effects may be produced by chemical materials set free from the genes that affect the cytoplasm as long as they are produced.

The embryologists, not being able to escape the formidable array of experimental data, on which the gene-theory of heredity rests, somewhat beg the question by saying:

"The complex of genes plays upon the organism at every stage; it acts thus upon the segregates and diversifies them in innumerable ways" (Lillie, 1929, p. 528). Or (Conklin, 1929, p. 30):

> We know that the organism consists of machines within machines. The inner machine in every cell is the nucleus, usually containing two sets of chromosomes and genes, any one set of which is capable of giving rise to an entire organism if it is not prevented by the outer machine consisting of cytoplasm and the products of differentiation.

From the foregoing it is clear that as now arrayed the two camps—of embryologists who adhere to the theory of embryonic segregation and of geneticists who uphold the gene-theory of segregation of genes—are hopelessly at odds. I propose to show in the following pages that this antagonism is unwarranted because there exists no real irreconcilability between the physiology of development and genetics.

But first permit me to affirm my acceptance of the data both of the physiology of development and of genetics. The established facts in each field of research where they have been amply confirmed and accepted call for no vigorous asseveration. I of course dismiss the patently spurious observation and the obviously incomplete and imperfect experiment and consider only the verified data constituting the body of knowledge.

With respect to the development of the animal egg, none doubts that the adult organism arises from the egg. Sperm-cells, as every other cell of the metazoon, originate from an egg. A spermatozoon alone never develops into an animal. An egg with or without the spermatozoon—*i.e.*, after fertilization or in normal or in experimental parthenogenesis—produces the adult. Only mature spermatozoa fertilize eggs; eggs are fertilizable as ovogones, as primary and secondary ovocytes and as ovotids. Thus, there can be no embryogenesis without egg cytoplasm. These are incontrovertible facts of classic, descriptive embryology. Whilst the experimental data of embryology are by no means as large or as significant as we sometimes enjoy to believe—deluding ourselves as we

seek to inspire in the uninitiated a belief that we have solved more problems than we actually have, and certainly in no measure comparable to the wealth of data accumulated by the indefatigable Drosophila-culturists—nevertheless, the information concerning merogony, development of isolated blastomeres, of the non-essential rôle of cytoplasmic inclusions, etc., constitutes a body of fact upon which as fact—omitting reference to interpretation—we can rely and which proves that descriptive and experimental embryology relate to the egg-cytoplasm.

We are also warranted in accepting the numerous and impressive data of genetics. Mendel's laws, being expressions of the statistical behavior of Mendelian characters, are true and would hold now as they did when first formulated if chromosomes did not exist. If anything were wanting to guarantee the validity of Mendel's laws, their rediscovery, where this was genuinely independent, as by Correns, for example, would remove all trace of doubt. But chromosomes exist. And in them has been found through the painstaking classical observations on mitosis a truly beautiful and precise mechanism to which Mendelian laws may be related. Dismissing as irrelevant the extravagant claims of the proponents of the gene-theory that their data are comparable to those of physics in quantitative nicety and exactness, we appreciate the dramatic in the experimental findings and accept these also as true.

What I reject is the proposition affirmed by the embryologists and tacitly admitted by the geneticists—namely, that the genesis of form and function and the genesis of particular characters in minute areas of the form are distinct and opposed. By development we designate the process by which out of one organism another comes into existence by means of the revelation of greater out of lesser complexity. By heredity we mean the fact that the offspring resembles its parent or parents. Though therefore *a priori* development could be thought of as showing no hereditary characteristics and hence we can not state categorically that the two terms, development and

heredity, are identical, nevertheless, all development which we know through experience shows without exception heredity, both at the beginning and at the end as well as at every intervening stage of development. The organism does not exist which has its genesis from an egg and at the same time fails to exhibit characters, Mendelian or otherwise. Nor is it possible for Mendelian characters, especially since they are minute and inconsequential, to display themselves apart from the form of the organism. An organism, either as egg, as adult or as any intervening stage of its differentiation, is heritage, inheritor and visible tangible evidence of heredity; its closest resemblance to its progenitor lies in its grossest and essential parts on which appear the inconsequential minor differences which constitute the Mendelian characters. Since neither process reveals itself independently of the other, we can postulate a cause common to both. I shall endeavor in this communication to prove that the same mode by which the grosser parts become differentiated calls forth the Mendelian differences. By overemphasizing the differences of the *terms*, development and heredity, biologists have overlooked the interdependence of the *phenomena* which these terms designate, have overlooked the fact that the physiology of development and genetics are but two aspects of the life-history.

In our attempt at the resolution of life, we abstract one aspect, the physiology of development, or another, genetics; but that we abstract, we should ever bear in mind. And the more we increase our knowledge of each by accumulation of exact and accepted data, the stronger becomes our duty to realize the unity of life. If our conceptions of these singly studied aspects of life—here of physiology of development and genetics—instead of expressing this unity run counter to it by opposing each other, we must abandon them and strive anew to encompass the facts of both domains by a re-interpretation.

The gene-theory of heredity as formulated by its upholders states that every cell in the body of the adult organism contains the same complex of genes—unchanged

descendants of those in the fertilized egg. The very unchanging character of the genes, while it may render the assumption that they control heredity apparently very easy, makes it difficult to ascribe to them a similar control in development which is so expressedly a series of manifold differentiations. When gene-theorists postulate that the genes order the progressive differentiation of development by liberating to the cytoplasm at different stages a different something which brings about the differentiation, they deal wholly in speculation without any basis of fact. While it is true that we are limited in our knowledge of biological processes, nevertheless theories should as far as possible be based on observed phenomena. Because of the lack of a factual basis we dismiss the gene-theory as an explanation of the physiology of development.

The current and increasingly popular theory of differentiation as embryonic segregation, however formulated by its individual upholders, I likewise dismiss. Whilst here I must limit myself to the discussion of one statement of this theory, that formulated by Lillie (1929), what I say against it applies in general to other formulations.

One general objection to the theory of embryonic segregation I base upon the use of the term, segregation. The term connotes preformation and its use by an avowed epigeneticist thus becomes confusing. Moreover, I can not understand why it is more "analytic" and less "vague" than the term, differentiation, since it relates to the progressive differences that arise in the course of the development of an egg into an embryo. Additional confusion arises through the correlation of segregation and dichotomy (*cf.* Lillie, 1929): the former implies separation of formed parts, "physiological molecules"—whatever these be—or potencies; the latter suggests branching into two. Either term, when united to the other, loses whatever meaning ascribed to it; together they explain nothing. Two substances may be segregated out of a system; dichotomy is by no means consequent to this segre-

gation nor is it implied in it. This leads to a further objection.

The visible dichotomy—cutting up into two—which can be followed during development is that of the cleavage of cells. No exact correlation exists between the cleavage of eggs and the postulated segregation of potencies. True, in eggs with so-called determinate cleavage, exhibiting cell-lineage, the dichotomy of the protoplasmic mass parallels loss of potencies by the blastomeres; but in other eggs, no such strict parallel can be noted. Further, by experimental means eggs of several worms, also of an ascidian, can be induced to develop to the swimming stage, albeit abnormal, without cleavage. The terms, segregation and dichotomy, can not therefore refer to a visible event during differentiation, the recurrent subdivision of the egg always by means of cleavage into two components. They are purely conceptual; as such they are open to these objections. But because of still more serious objections the theory of embryonic segregation must be dismissed.

Animal eggs are at first pluripotent. This is shown by the fact that fragments taken from unfertilized eggs (in the fertilizable condition) develop upon fertilization, whether the fragmented eggs be of the determinate or the indeterminate type of cleavage. Such fragments of eggs of *Chaetopterus,* for example, develop as well as those from an echinid egg. With first cleavage the former egg and with a later cleavage the latter lose pluripotency. This is brought out by the fact that their blastomeres develop defectively if they are separated in these respective stages. Thus eggs have originally the capacity to produce more than one embryo. According to the theory of embryonic segregation, this pluripotency means a plurality of segregates equal to the number of obtainable fragments capable of development—a number limited presumably only by the size of the smallest fragment that develops.[1] Let us now say that there are ten fertilization-

[1] The fact that fragments without cytoplasmic inclusions develop as well as those with them indicates not only that the cytoplasmic inclusions are

and development- capable fragments. Then there are ten sets of embryonic segregates present in the whole egg. If we take, for example, the egg of a sea-urchin or a worm, according to the theory of segregation there are segregated ten potencies for each structure to be. A human egg would segregate the potencies for ten heads, ten arms, ten legs, ten thoraces, ten abdomens and ten parts in each of these regions. Instead of potencies for twelve cranial nerves there would be 120. How would these stand in relation to each other? Would they coalesce as drops of mercury run together to form one drop? Or would they form polymeres? Ten head-potencies by polymerization becoming one—much as n numbers of $C_6H_{12}O_6$ molecules polymerize to form starch? But no such picture as coalescence or as polymerization is strictly speaking a segregation. Even if we suppose that ten possibilities for heads telescope on each other, we must farther suppose some change other than mere aggregation to bring about the result of the final single head.

Another objection to the theory of embryonic segregation is that it lacks any relation to a visible segregation-process. The only visible segregation occurring during development is that of the cytoplasmic inclusions and for these it has been sufficiently established that they are non-essential to cleavage and embryogenesis. They may be displaced by centrifugal force without impairment of the egg's development. Inclusion-free fragments of eggs having been fertilized develop as do whole eggs. No embryologist of to-day would postulate a theory of embryonic segregation on the basis of the altered disposition of these cytoplasmic inclusions, the only segregation observable during cleavage.

Finally, the theory of embryonic segregation is purely teleological. From the result of the embryonic process it draws conclusions concerning the events of this process

non-essential but also that since in two fragments of equal size, one with and the other without inclusions, there is less ground-substance in the former than in the latter, the developmental capacity is limited only by the amount of ground-substance present.

without paralleling these with the intermediate stages of differentiation. Development, to be sure, may be teleological. But merely to conceive it as such does not prove it such. The teleological argument becomes weak indeed if it goes against demonstrated facts. And this is the case here, as will become evident in the following presentation.

II. Nuclear Increase as a Factor in Differentiation During Cleavage

Although I limit myself in this discussion largely to the differentiation that occurs during the cleavage-process, I take the position that whilst we may adhere to the classic subdivision made by Karl Ernst von Baer, of the entire process of embryogenesis into stages, we should also keep in mind, as did von Baer, that these stages are by no means sharply delimited by hard-and-fast lines. Moreover, I hold that the process of differentiation is fundamentally the same, whether it be of germ-cells from somatic, of fertilized from unfertilized eggs, of cleavage, of germ-layers or of histogenesis. The origin and differentiation of the appendages of the skin make a problem which at basis is comparable to any that involves the revelation of potency and which therefore belongs to the whole domain of the physiology of development as surely as the cleavage-process. Differentiation is progressive; although for convenience we separate it into stages, we should remember that as it progresses it moves forward without any breaks despite changes in expression. Hence, although I here confine myself to that differentiation which occurs during cleavage, the argument in my judgment applies equally well to any other stage of the embryological process.

Whatever the stage in differentiation, we deal with cells. These have always fundamentally the same organization, that is, every cell is in itself differentiated. This differentiation, commonly and grossly regarded as one of nucleus and cytoplasm, I consider more exactly a differentiation of the basic ground-substance into nucleus on the one side and ectoplasm on the other. Speaking in

what follows of cytoplasm, I always mean the ground-substance, excluding nucleus and ectoplasm.[2] During cleavage two definite, visible and readily followed changes occur; these are the increase of ectoplasm and that of the nuclei. Both are factors in differentiation. It is to the increase of the nuclei that I wish to direct special attention.

Whilst with each successive nuclear division during cleavage of the egg, the nuclei progressively diminish in size, no individual nucleus in any blastomere at the end of cleavage ever vanishes. On the contrary, the total quantity of nuclear substance in an egg, where this has been determined, is greater at the end than at the beginning of the cleavage-period. The quantitative increase in nuclear substance continues through the whole embryonic period and becomes therefore a definite characteristic not only for cleavage but also for the whole course of development. The cells comprising the body of a newly hatched chick contain nuclear matter far in excess of that in the uncleaved egg which gave rise to the animal. Indeed, as long as the chick lives and any of its cells multiply, it elaborates nuclear substance. Moreover, every organism that regenerates lost parts, every one whose tissues exhibit pathological growths, as tumors, builds nuclear substance. Hence, the building up of the chemical constituents of the nucleus represents a basic property of protoplasm as a self-reproducing system. In other words, as protein-synthesis represents that fundamental chemical characteristic of living matter that distinguishes it from non-living, so nuclear synthesis (in part a protein-synthesis also) stands as one of the primary chemical activities of the self-regulation and the self-differentiation exhibited as special attributes of cells capable of reproducing themselves. The course of development is marked by syntheses. By these arise secretions as those of the thyroid, the pancreas, etc., and such compounds as

[2] I use the word, cytoplasm, in this meaning to avoid circumlocution. Beyond, when I give it broader meaning, I add the term, ecto-endoplasm, in parenthesis.

haemoglobin, as found in the embryos of animals which possess these substances. By synthesis also nuclei arise.

The question now is: Out of what are the nuclei synthesized? With the progressive increase in amount of nuclei runs during the period of cleavage a decrease in that of the cytoplasm without any change in the amount of the yolk and oil. This fact, holding for all animal eggs, is especially clearly shown in totally cleaving eggs. In the egg of *Nereis,* for example, I have followed very exactly the composition of the blastomeres throughout cleavage and can state that in the blastula-stage the total amount of cytoplasm is less than that in the egg at first cleavage, whereas the amount of oil and yolk does not change. The clearest evidence for the statement that nuclei arise out of the cytoplasm is furnished by the development of fertilized inclusion-free fragments of eggs, for in the cleavage-process of such fragments the nuclei also increase. Furthermore, it has been shown that the nuclei in yolk-free blastomeres are larger than those in yolk-rich blastomeres and that the size of the nuclei is proportional to the hyaline content of blastomeres rather than to the total, encompassing the inclusions (Conklin, 1912). These facts prove that in totally cleaving eggs during cleavage the nuclei are synthesized from the cytoplasm. Also in partially cleaving eggs the yolk takes no part in development during cleavage. When cleavage is ended in these eggs, new cells are added to the embryonic area from the yolk region. But even then the evidence indicates that the yolk is transformed into cytoplasm and does not go directly into nuclei. The increased nuclear content at the end of cleavage therefore can only mean that the nuclei are elaborated out of cytoplasm, since we can not postulate an automatic activity of the nucleus capable of building nuclei out of nothing.

Now what has been said of the nucleus as a whole applies equally to its constituents, the chromosomes. In a forthcoming publication I shall give evidence on which I base the conception of the nucleus as a composite structure, considering each chromosome as a single nucleus.

From this point of view, the debate concerning achromatin and chromatin, especially when based largely on staining reactions held to be specific for chromatin, already of little significance, becomes of less value. From the chromosome are derived all the morphologically discriminated structures of the nucleus—chromatin, linin, nuclear wall, etc. What we have regarded as discrete morphological elements of the nucleus, I consider as chromosomal in origin, their differences in structure being expressions of physical changes undergone by chromosomes in mitosis. Since during the differentiation-process of the fertilized (or parthenogenetic) egg the nuclei grow, the chromosomes grow. But quite apart from my conception of the chromosomes as each being a nucleus, some (though scant) evidence exists concerning chromosome-increase directly (Baltzer, 1908; Godlewski, 1908; Erdmann, 1909; Conklin, 1912, and others).[3] Let us then consider the history of the chromosomes themselves during cleavage.

At each cell-division the chromosomes are halved in quantity. Since, as we know, they never reach the vanishing-point, they must increase in mass during the period of cleavage. Says Morgan: "During development, especially during early cleavage, the amount of chromatin steadily increases in amount, giving an exponential curve resembling the first half of a curve of a monocatalytic reaction" (1927). What evidence we possess definitely warrants the statement that the chromatin material increases in amount during cleavage. The increased amount of chromatin during cleavage, as that of nuclei, is derived from the cytoplasm.

It is thus clear that the increased amount of nuclei and of chromatin is at the expense of the cytoplasm. Equally clear is the fact that with this total increase the cytoplasm diminishes. There is thus finally less cytoplasm as such, whilst the total mass of chromatin has increased. The growth of chromatin and the decrease of cytoplasm run

[3] For determination of nucleic acid in eggs see Masing (1912), Brachet (1933).

parallel with differentiation. This fact clearly leads to the assumption that differentiation to some extent hangs upon the taking up of cytoplasmic material by the chromosomes. Thus we deal in differentiation with the definite increase of a chemical stuff, nucleo-protein; a measured weight of this must mean a definite weight of precursors that produce it. Briefly, we are here concerned with the formation of a known chemical stuff paralleling differentiation.

I regard this building up of nuclei and chromosomes, that is, the synthesis of nucleo-protein, as one of the two factors which always accompany the differentiation of development. Indeed, this concomitance is so strict that it lies close at hand to postulate that in these factors lies the cause of differentiation. The other no less important factor, as already stated, is the visible, easily demonstrable increase of the ectoplasm. This second factor, with which I have dealt in other publications, I need mention here only in passing, since it does not so intimately concern the point at issue. That the ectoplasm is a factor in differentiation we must bear in mind in order to have a complete picture of the differentiation process.

In relating differentiation in part to a chemical process, we may have close at hand an explanation of differentiation as a series of chemical changes. In this wise it may be possible to detect certain chemical stuffs as potencies. Surely, the more we can substitute for such terms as potency chemical reactants in chemical reactions, the closer shall we come to the solution of the problem of differentiation of development. In relating differentiation in part to the synthesis of nuclear material, we take the first step in this direction.

I therefore consider that the progressive differentiation of the egg during cleavage is not the result of the pouring out of stuffs by the chromosomes into the cytoplasm, nor that of segregation of embryonic materials, but more truly the result of a genetic restriction[4] *of poten-*

[4] The term, genetic restriction, is an old one and was first used by Minot. In this article, however, this term for the first time is related to an actual event (process).

cies by the removal of stuff from the cytoplasm to the nuclei, the restriction being related in turn to the activity of the ectoplasm.

Consider a fertilized egg, *ABCD,* with determinate cleavage, which at first cleavage forms two blastomeres, *AB* and *CD;* there must be differentiation, since the *AB* and the *CD* blastomeres when separated give rise to partial larvae. This would mean according to the theory of segregation that *AB* is minus *CD's* material and *CD* is minus *AB's*. *AB* thus would be a cell in which the prospective *AB*-potencies are present; the same would hold for the *CD* cell for the *CD*-potencies. After the second cleavage, *A*-, *B*-, *C*- and *D*-potencies are present in blastomeres *A, B, C* and *D,* respectively. This would be true at each succeeding cleavage. For eggs with indeterminate cleavage the presence of material—and conversely, the loss of other material—would appear at that stage in the cleavage-process when the blastomeres on separation no longer possess the potency for development into complete though dwarf larvae. But is an egg-cell (in this case with determinate cleavage) $ABCD = AB + CD$, as we would express it according to the theory of segregation? Or can we say that $AB = AB + (-CD)$; $CD = CD + (-AB)$? Can we say that *ABCD* is really $AB + (-CD) + CD + (-AB)$ and do we gain thereby?

Restriction is, as I shall show, preferable to segregation, for it more nearly expresses what really happens. Restriction implies a loss, in this case of developmental potencies, without necessarily a rearrangement of materials. Segregation connotes new arrangement or sorting out of materials without any implication of change in them. If, as cleavage progresses, cytoplasmic potencies for embryo-formation are merely shifted to new positions, then reversal of the cleavage-process would be possible by reversal of the order of the cytoplasmic stuffs. Stages of differentiation then would be repetitive. If I knew of a case in which an adult organism, a larva, a blastomere or even an egg in late cleavage did return to the state of the uncleaved egg, I could appreciate the use

of the term, embryonic segregation. I do not wish to indulge in hair-splitting definitions; nevertheless, I must say that I for my part can not see how the potencies of an egg by mere acts of separation can account for differentiation. Other objections to the theory of embryonic segregation I have given above. Far simpler than the postulate of a mere transposed order of potencies is the assumption that as development proceeds the individual blastomeres lose potency.

Suppose that we assume that at first cleavage blastomere *AB* becomes such because it loses *CD*-material, and the *CD*-blastomere becomes such because it loses *AB*-material. The uncleaved egg, *ABCD*, is in reality $AB + AB$ to $AB_n + CD + CD$ to CD_n. A separation into *AB*- and *CD*-blastomeres would mean that *AB* somehow removes all *CD*-material and *CD* all the *AB*-material. This could take place through the absorption of *CD*-material by the nucleus of the *AB*-blastomere-to-be and of the *AB*-material by the nucleus of the *CD*-blastomere-to-be. The subtraction of cytoplasmic materials by the nucleus would take place with each mitotic cycle.

Genetic restriction on this assumption depends upon the transfer to the nucleus of certain materials from the cytoplasm, leaving others free. With each cleavage each nucleus fixes all material other than that which makes the blastomere what it is, *AB* or *CD*; *A* or *B*, *C* or *D*, etc., to the end of cleavage. Then the nucleus of cell *AB* is different from that of the *CD*-cell, since the *AB*-nucleus contains bound *CD*-material and vice versa for the *CD*-nucleus.

The potencies for embryo-formation, then, are all present in the uncleaved egg. Depending upon the species, the egg loses pluripotency, resident as far as we know in all unfertilized eggs, early or late after fertilization. The first step in restriction is this loss of pluripotency. By transfer the potencies for multiple embryo-formation to the nucleus (or nuclei)—at that cleavage-stage when blastomeres on isolation no longer develop as complete embryos—in the cytoplasm the potencies are left free for

the formation of a single embryo. In turn, during cleavage progressively these potencies are transferred to the nuclei and the blastomeres are more and more restricted.

We can relate differentiation to the transfer of stuffs to the nuclei, that is, to the increase of nuclear material, but this does not mean that increase of nuclear material as such indicates transport of potencies. For example, the tissue-cell of the adult, a cell no longer capable of differentiation whilst normally a part of the tissue and capable of multiplying, shows nuclear increase; nuclei here also are elaborated out of the cytoplasm, which in turn grows by intake of foodstuffs. And as long as such a cell multiplies, its daughter-cells normally maintain finally a certain size, characteristic for this tissue. We therefore must make the clear distinction between increase of nuclei and transfer of potencies to nuclei: the former—increase of nuclei—can proceed without involving transfer of potencies; the latter—transfer of potencies—is bound up with increase of nuclear amount. The study of nuclear increase both during and after differentiation, that is in differentiating and differentiated cells, and the comparison of the two processes will elucidate what in increase of nuclear material relates to the removal of potencies from the cytoplasm. Clearly, it is the loss of potencies, *i.e.*, the progressive restriction, that makes nuclear gain and cytoplasmic loss a factor in differentiation. The stuff transferred from the cytoplasm to the nuclei may be wholly or in part the potencies or the vehicle for them. Though we can not now determine definitely the relation of transferred stuff and potency, nevertheless, by correlating the transfer of stuff with removal of potency we have the basis for a more direct attack on the problem of differentiation than has been hitherto possible. For the first time we are able to localize potency in a definitely known complex of chemical stuffs.

The question now arises: When does the cytoplasm of the egg gain the potencies which later, during cleavage, are restricted? Since all eggs, for which we have data, before fertilization possess capacity for multiple embryo-

formation, this question becomes: When does the egg become pluripotent?

With the onset of the fertilizable condition, pluripotency becomes demonstrable, inasmuch as now fragments of the egg are when fertilized each capable of development. From this we might reason that the potencies that were stored up in the nuclei during the previous development of an egg are restored to the cytoplasm at this time, that is, to the cytoplasm of the new egg. However, the demonstration that pluripotency is present gives no evidence of the time when it arose. Merogony may be only an indicator of an earlier established pluripotency. Having no other criterion than fertilizability for the demonstration of pluripotency, we can not exactly define the moment when the egg becomes pluripotent.

An attractive hypothesis is that pluripotency arises with breakdown of the germinal vesicle when there escapes into the egg-cytoplasm residual nuclear stuff greatly in excess of what remains to form the mitotic complex of the first maturation-division. The potencies might be regarded as identical to or associated with this extranuclear material. According to this suggestion, the rise of pluripotency would be separated from the fertilizable period, for the fertilizability does not depend upon a particular stage in maturation. But since many species of eggs are fertilizable, whilst their germinal vesicle is intact, not a single one of these should be pluripotent if we are to relate the rise of pluripotency to substances escaping into the cytoplasm after breakdown of the germinal vesicle. On the same basis of reasoning, where fragments taken from an egg fertilizable in the stage of the intact germinal vesicle develop when fertilized, it must be ascertained that the fragments are devoid of stuff having escaped from the germinal vesicle. The data on merogony in such eggs, that we so far possess, do not warrant a decisive conclusion in these questions.

There remains another possibility. After the germ-cells have become differentiated from somatic through the loss to their nuclei of all potencies, with only the

potencies for germ-cells left free in their cytoplasm, they become isolated from the soma. This isolation brings about the escape of all potencies that were up to that time bound in the nuclei, into the cytoplasm. Thus they would become pluripotent.

The value of the theory of genetic restriction is brought out by the fact that it offers an explanation for each of the following phenomena: (1) polyembryony; (2) merogony; (3) development of diploid fragments; (4) development of isolated blastomeres; (5) asexual reproduction.

(1) The capacity of some eggs, as those of certain insects, normally to produce many embryos from one egg, and of other eggs, as those of the armadillo, to give rise always to several embryos from one egg, as well as the possibility of producing twins from normally monoembryonic eggs, including those of the determinate type, as the eggs of *Nereis, Chaetopterus, Tubifex,* etc., experimentally, shows that eggs have more latent potency than that required for producing one animal. A theory postulating a restriction of potencies is in greater accord with these facts than one suggesting that potencies for the embryo are segregated.

(2) This same conclusion holds for the data on merogony. It may be regarded as an experimental proof that all eggs which can be fragmented before fertilization have the capacity for the production of many embryos. Merogonic development indicates strongly, especially in determinate eggs, that differentiation ensues as a restrictive process—restricting potency for multiple embryoformation to one. The theory of segregation in these cases would imply an accumulation of like parts.

(3) In the development of diploid fragments, restriction obtains as in whole eggs, the chromosomes receiving less cytoplasmic stuff, since less is available. From the point of view of embryonic segregation, however, we would have to assume that, since perfect though dwarf embryos result from the fragments, the segregates in whole eggs are pluralistic aggregates. From this it

should follow that all eggs when separated into blastomeres should develop into entire organisms or into embryonic regions, each containing parts reduplicated.

(4) The development into perfect though dwarf embryos from blastomeres isolated during cleavage stands as strong proof that restriction underlies differentiation. The fact that potencies sooner or later are lost argues more for their removal from the cytoplasm than for their translocation in it, since the development of isolated blastomeres is the same as that of the whole egg. One would expect, according to the theory of segregation, that the segregates would take up new positions due to the new relations caused by isolation. According to the theory of restriction this factor does not enter.

In passing, I should like to refer to an objection which I have previously raised against certain conclusions drawn from experiments made on the development of the isolated blastomeres of echinid eggs (Just, 1928). First, in these experiments the vitelline membranes are removed from the eggs by squirting the eggs from a pipette—a method which I have found to be harmful. Moreover, the best time for such removal is that, as Boveri showed, at which the eggs are most susceptible to injury by mechanical shock, as manifested by their subsequent abnormal development—prolongation of the monaster stage with pronounced ectoplasmic deformation. My method of removing the membranes by putting the eggs through bolting silk is wholly innocuous. In the second place, the eggs in these experiments are further treated by being placed in calcium-free sea-water and thereby farther injured. Although Hörstadius (1935) in his recent monograph dismisses Peter's comment on the injurious action of Ca-free sea-water, we must consider it, since earlier workers have likewise noted this action. It is my judgment that any interpretation concerning the development of isolated blastomeres obtained from echinid eggs subjected to a twofold method of injury should be accepted with reservation, if not with scepticism. The less injurious and more direct means of separating blastomeres available should be used.

(5) Asexual reproduction can be explained by assuming that potencies in the egg, present in excess of those for the formation of a single individual, remain free. The reduplication of the animal by bud or fragmentation, though it occurs late in the life-history, is thus comparable to polyembryony. Where, as in *Obelia* and in other forms, alternation of generation occurs, egg- and sperm-producing areas as motile swimming organisms arise, restriction has taken place by removal of these free potencies.

According to one formulation of the theory of embryonic segregation the segregates are under the dominance of the nucleus (see quotation from Lillie given on page 270). Again I may mention certain phenomena which are better explained by the theory of genetic restriction than by this version of the theory of embryonic segregation.

(1) If we put forward the theory of segregation to account for the differentiation occurring in a parthenogenetic egg containing a nucleus with half the number of chromosomes characteristic of the species, we encounter the difficulty of explaining how under the dominance of this "half" nucleus the cytoplasm is ordered into regions whence the organs arise. According to the theory of genetic restriction, this difficulty does not obtain. Since we must assume that such eggs are stimulated to development during maturation, the polar bodies remove potencies so that the haploid nucleus is now in a less heavily laden cytoplasm. In experimental haploid parthenogenesis the same reasoning applies, whether the treated egg be one fertilizable (and thus stimulated) in the stage before or after complete maturation, for the experimental means induces in the egg-cytoplasm changes comparable to those following fertilization and hence brings about a restriction in potency.

(2) The same can be said for eggs developing with polyploid nuclei. In the egg of *Nereis*, for example, fertilized after treatment with ultra-violet light (Just, 1932a), the polyploid nucleus, made up of three nuclei from the suppressed polar bodies plus the egg- and the sperm-nuclei, receives potencies in precisely the same way as

the zygote nucleus in normal fertilization. But from the point of view of segregation conditioned by nuclear material or power escaping into the cytoplasm, development should not take place because of the overpowering influence of the superabundant nuclear matter.

(3) The capacity for regeneration, that finds no explanation by the theory of segregation, may be thought of as a reorganization under the influence of the liberation of potencies previously bound by the chromosomes into the cytoplasm induced by the changed condition of the cells as a result of the injury or loss. The origin of sex-cells from non-sex-cells in the regeneration of sexual animals which have been cut into two is similarly explained. Previously quiescent cells, whether somatic or "formative," become isolated and their nuclei freed of potencies, as normally those in egg-cells are freed after egg-cells have become isolated from somatic cells. In this connection a word may be said concerning sex-reversal.

Some years ago (1922) I obtained what appeared to be sex-reversal in *Platynereis megalops* (by cutting females with immature eggs into two) (Just, 1929). But I pointed out that I was sceptical of the result as a true sex-reversal because I was not positive that the life-history of my animals, raised in the laboratory from eggs, was identical with that occurring in nature: *P. megalops* might under natural conditions pass through a stage of hermaphroditism comparable to that described for *P.* (*Nereis*) *dumerilii* at Naples. So also since, as is well known from Korschelt's studies (1895), hermaphroditism obtains in *Ophryotrocha,* Braem's findings (1893) that regenerated pieces of this worm are male or female can not, strictly speaking, be regarded as a case of sex-reversal. Every case interpreted as one of sex-reversal demands most critical examination. But where it does obtain, to whatever extent, we can explain it as due to alterations in the cytoplasm called forth by changes (experimental or natural) in the environment.

(4) Abnormal growths or tumors can be explained in the same way. Some change in the environment of the

cells stimulates the throwing out by the nuclei of potencies into the cytoplasm, where a new type of development is set up. A tumor-cell is one which has escaped the domination exercised by contiguous cells. It becomes physiologically isolated.

During cleavage, the mass of nuclei increases, whilst the cytoplasmic mass decreases. The fact that in each mitotic cycle material escapes from the nucleus into the cytoplasm does not offset these changes in nuclear and cytoplasmic mass. With them the egg during cleavage becomes progressively restricted, as shown by the limitation in developmental capacity of the constituent blastomeres. It is thus logical to relate the loss of potency to the loss of cytoplasmic material to the nucleus. The hypothesis of genetic restriction as a factor in differentiation therefore is consistent with observed phenomena.

III. An Interpretation of the Rôle of the Gene in Heredity

In the preceding section I limited myself to the exposition of my theory of the rôle of the nuclear increase in development according to which I interpret the fact of nuclear synthesis out of cytoplasmic precursors as the means of a selective removal of potencies, with the result that in the cytoplasm other potencies remain free under whose action differentiation ensues. By using the indifferent terms "removal" and "transfer" I endeavored to avoid any implication that would ascribe the active determination of what is removed to either nucleus or cytoplasm. Up to the present we have no evidence that permits us to assign such hegemony to either cell-component. Our knowledge of cell-metabolism indicates that neither alone underlies the phenomena of life, but rather that these express themselves as an interaction of both. Thus we are at present not able to answer the question, which cell-component is the primary cause of the transfer of stuffs to the nucleus from the cytoplasm, but we can proffer an hypothesis that ascribes a definite rôle to the partners in the interaction. This hypothesis must be consistent both with our knowledge of the structure and

behavior of the cytoplasm and with the rich data accumulated by geneticists which relate to the structure and behavior of the chromosomes. Since these data have served as the basis for the elaboration of the gene-theory that ascribes hegemony to the gene, we must focus our attention on them and on this theory.

It is a fundamental postulate of the gene-theory of heredity that the genes maintain their integrity throughout the entire life-history. This postulate rests on the basis of cytological studies which indicate the maintenance of form and the constancy of number of the chromosomes. Since the theory further postulates that each cell receives the same genetic complex (*e.g.,* Morgan, 1927), we assume that the genes grow. As parts of the chromosomes they must grow as the chromosomes do, namely, at the expense of the cytoplasm, unless we assume for the genes magic powers. At the same time the gene is regarded to be the controlling power in the cell both in heredity and in development. Thus the process by which the genes increase in number and that by which they exercise control are in direct opposition to each other, according to the gene-theory; certainly, the theory does not relate these processes in any way. This conflict reveals one fundamental weakness of the gene-theory.

The gene-theory of heredity is an ultra-mechanistic, rigidly bound concept. Many biologists, among them especially physiologists and biochemists, are not yet ready fully to accept the preformationistic and vitalistic implications with which geneticists have endowed their gene as an entelechy—now no longer a vague principle but becoming phenomenal in the garb of non-physical conceptions of its physical behavior, a garb derived from a method of quantitative study which is mistakenly identified with the methods of analysis employed by chemists and physicists.[5] This ultra-mechanistic rigidity of the gene-theory renders it valueless for explaining the process of differentiation, a process marked by the egg's inherent plasticity and by its mobile responses to external

[5] See the statement on the very first page of Morgan's "The Theory of the Gene," 1926.

influences; it sets off too sharply the process of heredity from differentiation of development.

This same rigidity of the conception has made it difficult for the proponents of the gene-theory to give an interpretation of the rôle of the gene both in differentiation of development and in Mendelian inheritance. They confess ignorance as to how the genes act both in development and in heredity (Morgan, 1924, and elsewhere). They speculate concerning the possibility of gene-control manifesting itself in one or another phase of mitosis, always on the assumption that the genes pour out something into the cytoplasm—never allowing for the far more reasonable possibility that, as unchanging particles that grow, the genes may add to themselves substances from the cytoplasm. Speculation unsupported by fact is often interlarded with experimental data and supposition is supplemented by supposition as the situation demands. At critical points the theory breaks down. Because it so frequently extends itself too far into the realm of the unknown, it makes too great demands on credulity. Moreover, it often takes as a basis far too questionable evidence. As witness, take the following (Morgan, 1932, pp. 285–286):

> If another branch of zoology that was actively cultivated at the end of the last century had realized its ambitions, it might have been possible to-day to bridge that gap between gene and character, but despite its high-sounding name of *Entwicklungsmechanik* nothing that was really quantitative or mechanistic was forthcoming. Instead, philosophical platitudes were invoked rather than experimentally determined factors. Then, too, experimental embryology ran for a while after false gods that landed it finally in a maze of metaphysical subtleties. It is unfortunate, therefore, that from this source we can not add, to the three contributory lines of research which led to the rise of genetics, a fourth and greatly needed contribution to bridge an unfortunate gap. I say this with much regret, for, during that time and even now, I have not lost interest in this fascinating field of embryological experimentation. It is true that a great deal of factual evidence came to light, and it is true that many misleading ideas were set aside, but the upshot was negative so far as the formulation of any of the factors of development, whether mechanistic or otherwise, are concerned. This may be because the work was pioneer and largely qualitative. Perhaps my disappointment at the outcome of the work has led me to an overstatement of its failures. Something did emerge that the future may show to be of fundamental importance for genetics. I mean the experimental demonstration that the immediate factors in the differentiation of the embryo are, at the time of

their activity, already in the cytoplasm of the cell. Second only in interest was the discovery that, within certain limitation, the already determined specificity may be reversed, or rather, shall I say, the initial steps already taken are reversible by factors extraneous to the individual cells.

These statements call for further elaboration, because they are unconsciously in the background of much of our thinking about genetic problems, and should if possible be more sharply formulated.

That the form of cleavage of the egg is determined by the kind of chromosomes it contained before the egg reached maturity has been sufficiently proven; and since the foundations of all later differentiation are laid down at this time, the demonstration is of first-rate importance for genetics, because it shows that we are not obliged to suppose the genes or chromosomes are functioning at the moment of the visible appearance of characters.

This is demonstrated by introducing into the egg foreign sperm of a species having another type of development. Although the chromosomes from the sperm are present from the first cleavage onward, they produce at first no effect on the cleavage; only after a time do they succeed in bringing about changes in the embryo. This evidence is, as I have said, important for our genetic analysis, for it serves as a warning that the time relations between gene and cytoplasm may have a relation different from that of an immediate dynamic change in the cytoplasm. The preparation for the effect may have taken place long before the actual event.

Of this *ex cathedra* pronouncement, a veritable decree of authoritarianism, we may waive that part which refers to a founder of modern experimental embryology, for no student of this branch of zoology can in sobriety fail to recognize his debt to Wilhelm Roux. On his principles of Entwicklungsmechanik, as upon the Weismannian conceptions, however far in the light of newer knowledge we have supplemented or even supplanted them, rests much of experimental embryology to-day and of genetics even. I do call attention to the fact that Morgan uses the slender evidence from experiments on cross-fertilization in so fundamental a manner and with so far-reaching implications for the gene-theory. In my judgment, these studies are, in addition, about the most poorly controlled in all experimental embryology. Surely, it is well known that those very means which are used to induce cross-fertilization produce similar and sometimes identical effects when used on straight fertilized eggs. From no experiment on cross-fertilization can we derive conclusions as to the effect of the foreign spermatozoon in the absence of the most rigorous controls by way of exposing

the straight fertilized eggs to precisely the same means employed to induce cross-fertilization. There is no evidence whatsoever that the chromosomes determine the form of cleavage. Further, even were it demonstrated that foreign spermatozoa introduced into the egg have the effect claimed, we would still not be justified in drawing the far-reaching conclusions for the whole of genetics which Morgan draws.

In contrast to the conception of the gene-theory, my fundamental thesis is that the genes act by removing stuff from the cytoplasm. Let me hasten to say that by no means do I consider my conception a definite, final explanation of the data of Mendelian genetics—it is an interpretation. But in several respects it is superior to the gene-theory. First, it does not over-reach itself as does the gene-theory by extending itself too far beyond what is known; it needs fewer assumptions. Second, it is consistent with the known facts both of differentiation and of genetics. Third, it is far more physiological, less mechanical and is consonant with our knowledge of cell-metabolism. Fourth, it does not violate the principle that nucleo-cytoplasmic organization, the protoplasmic system, is a unit.

I see in the nucleus that cell-component which tends to maintain its change-resisting character. Then, despite the occasional occurrence of abnormal chromosome-garnitures in cells, both of the germ-track and in the soma, I deem it probable that the chromosomes and their genes maintain their identity or individuality. But since they grow at the expense of the cytoplasm, they must take up material that carries or assumes the characteristics of what is already present in them; their maintenance of identity must stand in the relation to the cytoplasm somewhat as that of end-product to the source which furnishes its upbuilding—as the building up of crystals in a supersaturated solution. It may be that chromosome-growth is more than analogous to the growth of such crystals. Be this as it may, the constancy of form displayed by the chromosomes throughout the life-history, maintained

without any abatement of their characteristic behavior, indicates that in some manner they attract to themselves the stuff of which they are made.

On the basis of the postulate that differentiation during cleavage arises as the result of the taking up on potencies by the nuclei, leaving free in the cytoplasm those that give the blastomeres their characters, an organ becomes such because in the cytoplasm of its cells are free those potencies which make it a special organ. Every cell in an organism becomes what it is because its cytoplasm has certain free potencies whilst its nucleus binds all other potencies. These latter act, if left unbound in the cytoplasm, as obstacles to the display of other potencies. Thus, the removal of potencies at the same time means removal of obstacles to cytoplasmic reactions.

My fundamental thesis is that all the differences—*i.e.*, differentiation—that appear during development rest upon cytoplasmic reactions. These are made possible through removal of obstacles by nuclei, hence, by chromosomes and genes. The nuclei by this removal release the activity of the cytoplasm in one direction. Then the genes act by removing impediments to cytoplasmic reactions. Let us examine this proposition more closely.

I begin with the assumption that with the onset of the fertilizable condition of the egg its cytoplasm has all the potencies whence arises the future organism. Indeed, as experiments on fragments of eggs obtained during this period show, such cytoplasm is pluripotent: it has capacity to produce several embryos. The chromosomes in such an egg are now freer to remove potencies from the cytoplasm.

Contrast this condition with that in the history of the spermatozoon: instead of possessing a richly laden cytoplasm, the spermatocyte's cytoplasm is poor. With the two rapidly ensuing maturation-divisions this poor cytoplasm is divided among four spermatids. The mature spermatozoon is therefore composed of even less laden cytoplasm. The chromosomes of spermatozoon and egg thus are alike as to their readiness to bind cytoplasmic

stuffs. The difference lies in the fact that the sperm-cytoplasm has practically nothing to offer, whereas the egg-cytoplasm has all—and more than—that is necessary for the genesis of one embryo. Thus both egg- and sperm-chromosomes are prepared for removing stuffs from the cytoplasm but only in the egg are these stuffs present.

As the result of restriction during development, every cell in the most complex organism finally has in its nucleus all the potencies bound except that one which makes the cell specific; it alone is free in the cytoplasm. The egg-cell, for example, as soon as it becomes differentiated from other cells of the body, is such because it has all potencies bound in its nucleus except that which makes it an egg-cell. And it is this potency in the cytoplasm which determines the future growth and differentiation of the egg to that moment when the potencies that were bound in the nucleus are thrown again into the cytoplasm. The sperm-cell likewise is such because in its nucleus are bound all the potencies except that which determines the sperm-character of the cell. This is free in the cytoplasm.

Precisely the same argument holds for those characters in the cells of organisms which constitute the distinguishing marks used by geneticists in their experiments. The Mendelian characters reside in the cytoplasm. The action of the gene consists in removing stuff from the cytoplasm, thereby liberating the cytoplasm-located factors of heredity. I bring now several examples to show that this conception of mine is wholly consistent with those data of genetics upon which the proponents of the gene-theory are in agreement.

If a spermatozoon from a *Drosophila* possessing pure red-eye fertilizes an egg of an also purely red-eyed female, in the eye-cells which show redness, the genes descended from the egg- and sperm-nuclei remove from the cytoplasm the hindrance to the reaction leading to redness. The "factor," redness, is resident in the cytoplasm and expresses itself because the genes remove stuff opposing this reaction. If on the other hand the sperm-chromatin is descended from a white-eyed animal and

the egg-chromatin from a red-eyed one, then the sperm-chromatin when in those cells which give the color to the eye removes stuff and this removal releases whiteness-reaction, and the egg-chromatin removes stuff the removal of which releases redness-reaction, the result is that the cytoplasmic reaction is now no longer $r + r = R$, as in the first case, but $r + w = R(w)$ when $R(w)$ gives the dominant red-eye color. A second example also concerns eye-color. Says Morgan (1924, pp. 727 *ff.*):

> It is sometimes said that the cytoplasm must be as important as the chromosomes, since no development is known except in the presence of cytoplasm, and by its activity. Whether the cytoplasm or the chromosome is or is not equally "important" is a matter that cannot be determined, and is of little consequence. The statement is an example of obscurantism rather than of profundity. What genetics has so far discovered that bears on this relation can be briefly stated. It is this. All the examples of heredity that have been sufficiently worked out show that all adult characters and most embryonic ones (not even excepting those at the beginning of development which are given above) are accounted for by the known behavior of the chromosomes. In other words, they "follow" the chromosomes regardless of the source from which the protoplasm comes. An example may make this clearer. A female fly with pink eyes produces eggs, which, if fertilized by sperm from a red-eyed fly, give rise to offspring with red eyes. Conversely, a female with red eyes, fertilized by a male with pink eyes, also gives rise to offspring with red eyes. It has made no difference whether the cytoplasm of the egg came from the red- or the pink-eyed stock. The failure of the cytoplasm to influence the outcome is even better shown by back-crossing the F_1 (heterozygous) fly from either of the last two crosses to a recessive pink-eyed male. Half the offspring are red- and half pink-eyed. The latter are identical in eye color with flies both of whose parents were pink-eyed. Here the egg cytoplasm has been produced in the presence of the dominant gene in one of the chromosomes, but this has no effect on the eye color, if, after extrusion in the polar body, the red-producing genes are lost. It is clear that whatever the cytoplasm contributes to development is almost entirely under the influence of the genes carried by the chromosomes, and therefore may in a sense be said to be indifferent.

Compare with this the simple interpretation that my conception offers. The offspring of the cross, red-eyed ♀ × pink-eyed ♂ or that of pink-eyed ♀ × red-eyed ♂, will have red eyes because in either case the genes take out of the cytoplasm pink- and red-factors, leaving red. Thus, the result is similar to that in the cross white red given in the example above. Why in either case the color is not intermediate, as obtains in certain crosses between

plants with red and white blossoms or between strains of black and white fowls to produce the "Andalusian blue" doubtless depends upon the differences in the chemistry of the pigments involved. This does not affect the issue, namely, that the result can be more easily explained on the assumption that the cytoplasmic reactions leading to redness are released by the abstraction of pinkness by the "red genes."

The back-crossing of the F_1 (heterozygous) fly of either cross (pink-eyed ♀ × red-eyed ♂ or *vice versa*) to a recessive pink-eyed male is amenable to the same explanation. The immature eggs here have red and pink genes; if the "red-producing genes" are lost to an egg after extrusion of the polar body, the egg-cytoplasm is left with "pink-producing genes" and a pink-eyed fly develops. We need only to say that the lost ("red") gene is pink-abstracting and the remaining ("pink") genes remove redness, leaving the reaction, pinkness, free.

Will the reader indulge me here for a moment? I should like to dwell on this quotation from Morgan because it is such an excellent example of the reasoning followed by the gene-theorists.

I point first to Morgan's statement that the "red-producing gene" has no effect if it is lost (!). Then the presence of a gene is necessary for its action. We judge its action by results obtained. But since we can obtain no genetic results whilst both "red-producing" and "pink-producing" genes are present in the egg, that is, before maturation and fertilization, we can not say anything whatsoever concerning the action of any genes until after fertilization. Important for the gene-theorist here are not the stages before maturation of the egg but that stage when occur the changes necessary for his theory. Morgan in making a statement concerning the action of genes which are lost before maturation involves himself in serious difficulties arising out of loose thinking. But reflect on a greater offense against logic.

The cited passage, as many another that can be taken from Morgan's writings, contains a curious fallacy. The

examples are given to show the failure of the cytoplasm to influence the outcome. But already in the presentation of the examples, Morgan tells us that the egg-cytoplasm has been produced *in the presence of the dominant gene.* When he then says that it is clear that whatever the cytoplasm contributes to development is almost entirely under the influence of the genes, he reaches no conclusion but merely restates the earlier sentence. Since he puts this forward as fact, there is no point to his examples as proof of it.

In dismissing this reasoning, I do not dismiss the examples. I accept the data, I reject the interpretation given them. In my conception the chromosomes play a rôle in heredity. So does the cytoplasm. In the examples cited, according to my view, the hereditary factors for pink- or red-eye are located in the cytoplasm and pink- or red-eye results, depending upon the abstraction of red or pink respectively by the genes. This interpretation assigns clear and definite rôles to nucleus and cytoplasm in heredity. Neither is self-sufficient, but it is in the cytoplasm that the hereditary factors reside.

According to my conception, sex-determination also is cytoplasmic.

Whilst in the majority of animals the male is heterozygous for sex, in some others the female is. Further, where the male is said to be heterozygous, his chromosome-garniture may contain XO or XY, Y being markedly unequal in size to X; in some cases, although no differences in the so-called sex-chromosomes can be ascertained, they are assumed to be present. In other words, it can not be said that one sex is always heterozygous, nor can it be said of animals generally that the presence of an additional chromosome means "femaleness."

The egg-cytoplasm of all species in which the male is heterozygous for sex, I conceive to be more strongly male than female. When eggs of these are fertilized by "male-producing spermatozoa," the chromosomes take out femaleness and leave maleness free in the cytoplasm—this whether the male chromosomal garniture contains XO or XY (Y denominating a difference not necessarily

in size to X). In other words, nothing less than XX suffices to remove all "maleness" from the cytoplasm. In those cases where the female is heterozygous for sex, the egg's cytoplasm is more strongly female than male; then only the full complement XX will remove "femaleness" from the cytoplasm.

In *Drosophila* the male is heterozygous for sex, its egg-cytoplasm more strongly male. Then the explanation of "supermales" and "superfemales" is very clear according to my conception. So also the occurrence of haploid males in forms in which the male is heterozygous for sex.

Finally, I conceive that normal hermaphroditism is due to equality of maleness and femaleness in the cytoplasm of the egg. The chromosomes here take out maleness and femaleness, leaving respectively femaleness and maleness free. Where abnormally hermaphroditism occurs, gynandromorphs and intersexes arise, I postulate that the cytoplasm has become so disturbed that the normal removal by the chromosomes does not follow.

Sex can not be placed in the same category with eye-color, wing-form and other characters displayed by *Drosophila*-mutants. Sex pervades the whole organism. It is often recognized by anatomical so-called secondary, sexual characters and by physiological differences expressed by metabolism, as Riddle's elegant studies have shown. Nor can the gonads, where reside the sex-cells, the fundamental diagnostic of sex, be regarded as comparable to organs, as eyes, wings, etc. There is nothing to indicate that the mutant-characters as such, *i.e.*, as eye-color, etc., in *Drosophila* exercise the influence on the animal that sex does; if a white-eyed male is weaker than the normal red-eyed fly, if "supermales" and "superfemales" are even more degenerate than other mutants, tending more in the direction of the climax of the *Drosophila*-mutations—the lethal—we do not predicate the varying degrees of descent to the moribund upon these characters in the same sense that we predicate differences in metabolism upon sex. And to proffer the suggestion that in normally hermaphroditic organisms the ovary

arises at one level and the testis at another "referring such cases to the same process that determines in the development that one region of the embryo produces one kind of organ and another region a different kind of organ in the presence of all the hereditary factors" (Morgan, 1924) is not only beside the point—it is downright fallacious biology. Waiving the facts that there exist ovotestes and that germ-cells may or may not arise *in situ*, I need merely call attention to the fact that gonadectomy proves that the gonad is not to be compared indiscriminately with other organs of the body. These statements, generally appreciated by mere biologists, the gene-theorist as generally overlooks. But let me not be misunderstood: sex is inherited—as is the whole organism, as every one of its parts, including the inconsequential characters displayed by mutants. The basis of inheritance is always the same: it is the egg-cytoplasm from which develops the organism—every cell of it and its every characteristic.

Thus my conception, whilst consonant with the experimental findings of Mendelian genetics, places the determination of characters in the cytoplasmic reactions. The active "factors" for Mendelian characters do not reside in the genes. Rather, the genes by extracting definite materials from the cytoplasm render possible the reaction of the cytoplasm-located heredity factors. Only inasmuch as they take out substance do the genes determine heredity. My conception differs from the gene-theory chiefly in two respects: by locating the factors for heredity in the cytoplasm instead of in the genes and by offering an interpretation of "how the genes in the chromosomes produce the effects in and through the cytoplasm" of which Morgan confesses ignorance (Morgan, 1924, p. 728; 1926 (b), p. 26; 1932, p. 285).

IV. General Significance of Nuclear Increase During Cleavage for Cell-Biology

That out of the egg develops the adult both biologists and geneticists agree. Both appreciate the necessity of

the egg-cytoplasm in embryogenesis. Thus, some geneticists hold that the egg-cytoplasm furnishes only the body-form and make-up—which must include physiological processes also—and in saying this they admit that it is the cytoplasm that builds the embryo. What are most important for a *Drosophila* are eyes, legs, wings, abdomen, thorax—not eye-color, extra legs, vestigial wings, color of abdomen, bristles on the thorax. The cytoplasm makes organs; then it makes the characteristics of these organs. Differentiation proceeds by a restriction brought about by the nuclei which as they grow remove stuff from the cytoplasm, thus making possible cytoplasmic reaction. The gene acts in the same way: by removing stuff it frees the cytoplasmic factors in heredity to express themselves.

This then is a far-reaching conclusion which we draw from the fact that nuclear increase parallels cleavage. This visible measurable change becomes a tangible event to which we relate both differentiation and heredity. Our theory of differentiation and of the rôle of the gene in heredity is consistent with the direction of this change of amount of nuclear and cytoplasmic material. It postulates nucleo-cytoplasmic interaction; it recognizes the cell as unit and differentiation and heredity as aspects of development. Nuclear increase appears as the key-reaction of cell-metabolism in development and may very well be such in all cells capable of reproducing themselves. Thus it has significance for all biology.

A predominant chemical characteristic of protoplasm is its protein-content. To protein-structure we relate specificities, whether these concern either animals and plants as species or those as yet chemically vaguely known humoral reactions revealed in immunology. A species of organism is such because of its protein-composition and one immunity-reaction differs from another largely because of the proteins involved. The enormous number of possible amino acids that enter into the structure of proteins is more than sufficient to account for the differences encountered among living organisms.

But this is not to say that proteins alone make up living things. No protoplasm exists free of lipins and carbohydrates. Doubtless living protoplasm is a lipin-pervaded system owing its integrity in part to this condition. Sugar and its photo-synthesis are indispensable for the maintenance of the living world; in one form or another, carbohydrate is found in all cells. Further, they enter into many reactions: both lipin and carbohydrate play rôles in immunity. Life can not exist without water. There is no life apart from electrolytes. These constituents, varying quantitatively as well as qualitatively, also enter into protoplasmic constitution and thus in part may account for the diversity in chemical constitution of living things. Important as proteins are for specificity, they alone do not comprise the chemical substratum of life. A living thing is a complex of proteins, lipins, carbohydrates, water and electrolytes.

Nevertheless, it is the proteins which have received most attention from biologists. In the light of the history of protein-synthesis it is all the more remarkable therefore that the synthesis of nuclein as a conjugated protein has not been studied more extensively. The foregoing presentation, by emphasizing nuclear increase as a factor in differentiation, appeals for a more concerted study of this increase by biological chemists and by embryologists.

Our chemical conceptualization of the cell will become sharper through more accurate knowledge of nucleoproteins and their synthesis during cleavage. More exact data concerning the conversion of inclusion-free cytoplasmic ground-substance into nucleo-protein may give us precisely that basis for the chemical analysis of protoplasm which we need. Consider for a moment the composition of nucleo-protein. Protein is present as a base and in the nucleic acid always carbohydrate (sugar) is present. To what extent electrolytes (except calcium) enter into the composition is not well known. Lipin may be present in the living compound, being removed by the method used to obtain nucleic acid which employs boiling water and strong alcoholic solutions, etc., to destroy the

nucleases. Nucleic acid also contains phosphoric acid, the source of which may be lipins. Thus, the synthesis of nucleo-protein may represent one involving all the constituents of protoplasm. And if it involves protein and carbohydrate only, it may point more exactly to the rôle of each of the three organic compounds in protoplasm. Thus, a biochemical research of the utmost importance is here proposed, a research on the progressive building up out of cytoplasm devoid of inclusions—*i.e.*, out of the hyaline cytoplasmic ground-substance—of the nucleic-acid-protein-base-complex, nucleo-protein.

It is astonishing that another group of biologists, the embryologists, have so largely neglected the implications for their specialty which inhere in the progressive accumulation of nuclear material elaborated out of the cytoplasmic ground-substance and which parallels the differentiation during cleavage that they seek to understand. True, its value for embryology has been mentioned, as by Loeb (1907); but standing as it does as one of the tangible positive events of development, it has never received adequate attention. The observational studies on the amount of nuclear material built up during normal cleavage are significant only for their paucity. Experimental studies, data on eggs subjected to treatment, almost do not exist. Whereas there lies at hand this definite fact, that with cleavage nuclei increase in number and their substance in amount, embryologists, especially the strictly morphological, content themselves with vague general statements to the effect that development means a chemical process, thus affirming what we take as a matter of course—namely, that living things, being material and not supernatural, have chemical organization. Or they postulate segregates, whether as "physiological molecules" or as some other material entities in the cytoplasm in contrast to gene-segregates. But such a segregation of stuffs or of potencies runs counter to the demonstrable fact that with development the cytoplasm loses itself in the gain of nuclei. For the embryologist as for the biochemist the imperative demand is the farther and

more complete study of the growth during development of nuclei out of the cytoplasmic ground-substance.

Let me hasten to add that in thus emphasizing the ground-substance I do not mean to imply that it is the living substance *per se*. It may have been it once—that is a matter for speculation. For us who deal with cells, especially eggs, living matter reveals itself as a nucleo-cytoplasmic organization. Though nuclei are synthesized out of ground-substance, we do not know that eggs exist as composed of pure ground-substance only. This, the ground-substance, is still too greatly unknown. Here again is indicated a research for both biochemist and embryologist. This is all the more true since the one school of biologists which by its own definition of its field of investigation should concentrate on the ground-substance, has instead grossly neglected it. I refer to biological colloid-chemists.

By definition colloid chemistry concerns itself with colloid solutions, *i.e.*, those whose particles range in size from 1 μμ to 250 μμ and which are mostly in the region of ultra-microscopy and in the upper ranges border microscopy. That there is some difference of opinion as to the upper size-limit of the colloidal particle does not affect my argument. This is that the vast majority of work on the so-called colloid-chemistry of protoplasm deals with the behavior of bodies in eggs that are visible under fairly low power of the microscope; it concerns itself not with what it defines as its domain but with the coarse gross formed bodies, as oil-drops, yolk-spheres, pigment granules and mitochondria. At best a border-science of questionable import because it is a catch-all of physics and chemistry, it further depreciates its value by failing to investigate its defined field.

But it is the physiological aspect of the fact of nuclear increase during cleavage that I wish to emphasize most. Here I use the term physiology in its broadest meaning to encompass all activities of the living thing, but in reference to cells only. It is often stated that the nucleus controls metabolism, dominates or initiates vital activities. Says McClung (1924, p. 634):

Taken together the chromosomes represent the sum total of all the elements of control over the processes of metabolism, irritability, contractility, reproduction, etc., that are involved in the life of an organism, but in the measure that organisms differ, so do the natures of their controlling mechanisms. These differences must extend over to the ultimate units of structure and the chromosomes, as aggregates of these, are at once an expression of the underlying unity of vital processes in all living things and an index of their specific and individual variations.

Such statements are absolutely without foundation in fact. To be sure, cytoplasm without nucleus is incapable of anabolism; but as surely, a nucleus without cytoplasm is inert. The spermatozoon, a cell with the greatest amount of nuclear substance known, nevertheless contains cytoplasm. Its short span of life is well known. To postulate that all physiological processes reside in the chromosomes is to over-reach fact, since no one single such process can be definitely assigned to them. It would be otiose here farther to dilate on this point.

The protoplasmic system, nucleus and cytoplasm (ecto-endoplasm), is structurally the biological unit and acts as such. Then physiological processes inhere in the interplay of the components of this unit, parts of an integrated whole. One approach to the understanding of the mode of nucleo-cytoplasmic interaction is indicated by study of the steadily increasing amount of nuclei and the in proportion greater decreasing amount of cytoplasm which parallel cleavage—the reason for the disproportionate decrease appears beyond. At basis, physiological processes signify chemical change. Hence, the initiation, course and end-results of chemical reactions in living cells should always be sought in order that the altered physiological state be evaluated. With respect to even the most simple reactions in the chemistry of non-living matter the chemist is free to confess that he has much to learn; chemical reactions in the living cell, their origin, duration and end-products, present a larger problem. When, therefore, we have alongside a demonstrable visibly quantitative chemical change, a synthetic reaction, involving a chemical compound as well known as nucleo-protein, we have

the opportunity to extend our knowledge not only of the embryological process but of the whole domain of what we denominate vital phenomena. We may postulate the presence of vital units, bio-gens, genes and the like, invoke the presence of hormones and enzymes—as if but to mention these give explanation;[6] they leave the problems unsolved. The growth of nuclei during cleavage, on the other hand, is the observable expression of the cell's maneuvers. The study of the chemical reactions involved has a significance for the whole domain of physiology which can scarcely be too greatly insisted upon.

More than once I have put forward the view that the cytoplasm is the seat of vital reactions (Just, 1928, 1931): that in fertilization the reaction is of the egg-cytoplasm; that the size of the centrosome-aster-complex in eggs fertilized with fractions of the spermatozoon is not determined by the amount of sperm-chromatin involved (Just, 1932a); and that the cause of the origin of mutations lies in disturbed cytoplasmic reactions (Just, 1932b).

To my view that the cause of the origin of mutations lies in the cytoplasm, Plough and Ives, on the basis of insufficient and inconclusive data, have objected. Even were their experimental data more complete, their interpretation of them would not hold. Thus, their answer to my argument, an argument based on a larger view of experimental cytology, carries no weight. Say Plough and Ives (1935, p. 61):

> Our data show that the tendency to produce an increased number of somatic modifications following heat is carried over by females only, while the increased rate of mutation is carried over by both males and females. The female gametes have large amounts of cytoplasm, while male gametes have little or none. There is no difference in the chromatin. Were the mutations always preceded by cytoplasmic modifications, the increased mutation rate should appear among the offspring of females only. Since it appears in the offspring of both sexes, we may conclude that the effect of heat is directly on the chromatin. Thus heat appears to affect both cytoplasm and chromatin independently.

Thus we are asked to believe that when flies are exposed to a temperature of 36.5° C for 24 hours, the heat pene-

[6] Loeb, 1916; Goldschmidt, 1927.

trates the entire organism in such wise that during the whole period of exposure it affects the chromatin directly. Even if *mature* spermatozoa isolated from the animal were subjected to heat, this conclusion of Plough and Ives would not follow; demonstrable cytoplasmic changes preceding nuclear might nevertheless take place. Certainly, physiological changes known to occur in animal spermatozoa after exposure to altered environmental conditions are related to their cytoplasm, small in amount though this is. Such physiological changes as decrease in locomotor activity and loss of energy are cytoplasmic; the tail (in flagellated spermatozoa), not the genes, moves the spermatozoon. These changes influence fertilizing power. If subsequently, on the other hand, it should be shown that heat affects the chromatin of a mature spermatozoon directly, my suggestion would not be vitiated. Such injured sperm-chromatin would be less capable of taking out cytoplasmic stuff in the egg than uninjured; the mutation arising would still express a changed condition of the cytoplasm. I emphasize mature spermatozoa, for Plough and Ives make a point of the presence or "little or no" cytoplasm and thereby exclude from consideration earlier stages in spermatogenesis when the cells have more cytoplasm.

The biological literature affords abundant evidence of the demonstrable physical and morphological effects of high temperature on cytoplasm generally. Both high and low temperature alter, for example, the physical state of the colloids. It thus becomes necessary to know precisely what are the effects of the exposure to 36.5° C. on sperm-cells within the intact fly, as compared with the effects of this temperature on other cells before we can consider Plough and Ives's conclusion. Finally, that "heat appears to affect both cytoplasm and chromatin independently" does not follow from their experimental results.[7]

This quotation from Plough and Ives exemplifies clearly the geneticist's conception of a living organism.

[7] In passing, I may point out that Plough and Ives's statement of their method can not be called lucid.

For the biologist a *Drosophila* is a member of the Class Insecta, Order Diptera, having certain structural characteristics which set it apart in the genus. Plough and Ives, the *Drosophilists,* as they call themselves, conceive a male *Drosophila* as an ambulatory complex of genes.

Let me repeat that I hold the view that the protoplasmic system is a unit and that I do not at all imply that the cytoplasm is self-sufficient. Rather, the cytoplasm is the field wherein the reactions take place. It may very well be that many of these depend upon the reaction by which nucleo-protein is synthesized out of the cytoplasm.

Again I say that life is a unit and life-processes can not be divorced from each other. My conception of the physiology of development and of genetics does not unite "two independent variables of the life-history"; these are never apart except by misconception and faulty abstraction. My conception does not unite—it recognizes a union which already exists.

More, it replaces conceptions predicated either upon antagonism between nucleus and cytoplasm or upon the dominance of the one over the other—conceptions which few biologists can reconcile except by such catch-phrases as, "heredity is effected by the transmission of nuclear preformation which in the course of development finds expression in a process of cytoplasmic epigenesis" (Wilson, 1925). My conception denies the "almost magical" power ascribed to the gene and robs gene-action of the supernatural and hyperphysical powers by recognizing the rôle of genes as within the domain of physiology. Thus, genetics becomes a branch of biology.

Above I said that as the nuclei increase during cleavage, the cytoplasm diminishes in greater proportion. Earlier I pointed out that nuclear increase is not the only visible concomitant of cleavage and differentiation; the superficial cytoplasm, ectoplasm, also increases. This ectoplasm-growth is responsible for a further loss of cytoplasm during cleavage. Both nuclei and ectoplasm are elaborated out of ground-substance. Physiological proc-

esses in living cells are related to chemical reactions within the cytoplasm; physiological processes which concern living cell and environment are related to the ectoplasm. By its powers of contraction and conduction, by its rôle in water—and gas—exchange, the ectoplasm responds to the outside world upon which it impinges. It also conditions the cytoplasmic reactions by circumscribing their field as cleavage advances; as the egg is subdivided progressively into capillary chambers of increasingly smaller dimensions, the result is increased surface-area with consequent alteration of reactions in which surfaces are involved. In addition, the ectoplasm is alive, a continuation of the protoplasmic system, and by display of activity which varies from moment to moment further modifies the cytoplasmic reactions. Elsewhere I shall deal more extensively than hitherto with the ectoplasm and its behavior; here I mention it to complete the picture.

The ever-changing ectoplasm in contact with the environment, on the one side; the chromosomes, static, change-resisting, on the other; the ground-substance between whence both ectoplasm and nucleus arose—this is my picture of the morphological unit whose physiological processes, by which we recognize the state of being alive, we seek to unravel. Nucleus and cytoplasm (endo-ectoplasm) render the cell a stable structural unit. Interaction between nucleus and cytoplasm gives physiological stability, equilibrium; the nucleus frees the cytoplasm to further activity. At the cell-surface, the ectoplasm conditions cytoplasmic activity. Always the protoplasmic system is the unit of life. And where we trace the development from egg to adult, we see that differentiation is inherited and heredity is a part of differentiation.

V. Summmary

(1) It is held that the apparent irreconcilability between physiology of development and genetics does not mean that they actually are separate. They are regarded

as two aspects of development and the data of both are accepted. However, the now more prevailing theory of embryonic segregation, and with it all theories of embryonic segregation, and the gene-theory of heredity are rejected as explanations for the physiology of development.

(2) Instead of any theory of a segregation, a theory of genetic restriction is proposed, in which it is postulated that the egg from its fertilizable condition, when it has capacity for the production of many embryos, is steadily restricted in its potencies. From a condition of pluripotency it becomes unipotent; then the blastomeres of this unipotent system with first cleavage or thereafter, depending upon the species of egg, become farther restricted and now give rise only to certain areas of the embryo. The loss of potencies is correlated with the increase of nuclear material. This increase is due to an elaboration of nuclear stuff out of the cytoplasm, that is, the ground-substance, whereas cytoplasmic inclusions directly play no part in this process. Since with this increase of nuclear material and the decrease of the cytoplasm that furnishes this material runs the loss of potencies, it is concluded that these processes stand in relation to each other. Hence, differentiation during cleavage is the result of a restriction brought about with the removal of material from cytoplasm to nuclei. On the basis of the chromosomal make-up of the nuclei, the removal is regarded to be by the chromosomes.

(3) The genes as components of the chromosomes are held to act in heredity as do the chromosomes in genetic restriction during differentiation. Then the gene does not act positively as carrier of the factors of heredity. Rather, these are all located in the cytoplasm. The genes function by removing material and thus free the cytoplasm-located factors of heredity to express themselves. This conception offers for the first time an interpretation of the rôle of the gene. For the examples given, this conception furnishes a better interpretation than that fur-

nished by the geneticists. The interpretation which the conception suggests for sex, haploidy, normal hermaphroditism, intersex-conditions, etc., is far more simple and direct and more consistent with the observed phenomena than any suggestion so far proffered.

(4) The conception of nuclear increase out of the cytoplasm as a factor in differentiation relates development to cytoplasmic reactions. On the other side, differentiation is marked also by an increase in the amount of the ectoplasm. To nucleus, cytoplasm and ectoplasm the conception assigns more definite rôles in development than other theories have ascribed to them.

The implications of nuclear increase as a factor in differentiation are discussed from the points of view of biochemistry, embryology and physiology. The conception by including the gene and by ascribing physiological action to it, emphasizes that the physiology of development and heredity are merely two aspects of the life-history.

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ADDENDUM

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